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- (71) Applicant (*for all designated States except US*): MAXY-GEN, INC. [US/US]; 515 Galveston Drive, Redwood City, CA 94063 (US).

- (72) Inventors; and  
(75) Inventors/Applicants (*for US only*): GIVER, Lorraine, J. [US/US]; 2538 Hawkington Court, Santa Clara, CA 95051 (US). MINSHULL, Jeremy [GB/US]; 842 Hermosa Way, Menlo Park, CA 94025 (US). VOGEL, Kurt [US/US]; 4151-D El Camino Way, Palo Alto, CA 94306 (US).

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(54) Title: NOVEL LIPASE GENES

(57) Abstract: New lipase enzymes (both nucleic acids and polypeptides) are provided. Compositions which include these polypeptides, proteins, nucleic acids, recombinant cells, as well as methods involving the enzymes, antibodies to the enzymes, and methods of using the enzymes are also provided.

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<b>NOVEL LIPASE GENES</b>
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**CROSS-REFERENCES TO RELATED APPLICATIONS**

Pursuant to 35 USC §119(e), this application claims priority to and benefit of U.S. Provisional Patent Application Serial Nos. 60/217954, filed on July 13, 2000, and 60/300378, filed on June 21, 2001, the disclosures of each of which is incorporated herein in  
5 their entirety for all purposes.

**FIELD OF THE INVENTION**

The present invention relates to the generation of novel lipase genes and homologues and to methods of recombination to produce novel lipase genes.

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**BACKGROUND OF THE INVENTION**

15 Lipases are enzymes which are involved in the breakdown of fats. Lipases are commercially important enzymes which have many current uses, including as reagents in food preparation processes (e.g., as additives to animal feeds), industrial degradative processes, crop engineering and even as treatments for several human diseases (e.g.,  
20 indigestion and heartburn (e.g., for pancreatic insufficiency), secondary cystic fibrosis, Celiac disease, Crohn's disease, obesity, etc.). The activities and sequences of several hundred lipases are known. See, e.g., [www.led.uni-stuttgart.de/](http://www.led.uni-stuttgart.de/).

Because lipase enzymes are of considerable commercial value, the identification and development of new lipase enzymes is desirable. The present invention  
25 relates to new lipase proteins and nucleic acids, e.g., having novel sequences and activities, as well as variants thereof.

### SUMMARY OF THE INVENTION

The invention provides lipase polypeptides, nucleic acids encoding the polypeptides, antibodies to the polypeptides, and uses therefor; data sets containing character strings of lipase homologue sequences and automated systems for using the character strings as well as other functions that will be apparent upon further review. The present invention  
5 also provides *methods of producing modified lipase polypeptides.*

Various aspects of the current invention comprise an isolated or recombinant polypeptide comprising a sequence having at least 97% amino acid sequence identity to any one of SEQ ID NO: 75 to SEQ ID NO: 108. Such polypeptide can optionally comprise or  
10 exhibit lipase activity (e.g., it can degrade geranyl butyrate or neryl butyrate or both). Additionally, such polypeptide can exhibit enantioselectivity for geranyl butyrate over neryl butyrate. Such polypeptide that exhibits enantioselectivity for geranyl butyrate can comprise a sequence selected from: SEQ ID NO:76, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:86, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:104, SEQ  
15 ID NO:107, SEQ ID NO:108, SEQ ID NO:78, SEQ ID NO:87, SEQ ID NO:100, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:88, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:103, or SEQ ID NO:106. Alternatively, the polypeptide can exhibit enantioselectivity for neryl butyrate over geranyl butyrate. Such polypeptide that exhibits enantioselectivity for neryl butyrate over geranyl butyrate can comprise a sequence selected from: SEQ ID NO:81, SEQ  
20 ID NO:82, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:105, SEQ ID NO:84, SEQ ID NO:91, SEQ ID NO:92, or SEQ ID NO:93.

Furthermore, the polypeptide can comprise a polypeptide encoded by a polynucleotide sequence which hybridizes under highly stringent conditions over  
25 substantially the entire length of a polynucleotide sequence selected from SEQ ID NO: 1-54 (or a complementary sequence thereof), or by a polynucleotide sequence encoding a polypeptide sequence selected from SEQ ID NO: 55-108 (or a complementary sequence thereof), and wherein the polypeptide comprises one or more of: Lys at position 1; Thr at position 14; Ser at position 17; Arg at position 22; Glu at position 26; Pro at position 31; Gly  
30 at position 33; Glu at position 34; Pro at position 35; Pro or Thr at position 37; Ser or Lys at position 41; Gly at position 42; Arg or Glu at position 43; Ala at position 61; Tyr at position

75; Gly at position 96; Ser at position 97; Thr at position 104; Ser at position 107; Ala at position 125; Gly at position 129; Val at position 134; Cys at position 138; Lys at position 141; Lys at position 146; Thr at position 156; Met at position 160; Arg at position 166; or His at position 177. Alternatively, the polypeptide can comprise one or more of: Lys at position 1; Thr at position 14; Ser at position 17; Arg at position 22; Glu at position 26; Pro at position 31; Gly at position 33; Glu at position 34; Pro at position 35; Pro or Thr at position 37; Ser or Lys at position 41; Gly at position 42; Arg or Glu at position 43; Ala at position 61; Tyr at position 75; Gly at position 96; Ser at position 97; Thr at position 104; Ser at position 107; Ala at position 125; Gly at position 129; Val at position 134; Cys at position 138; Lys at position 141; Lys at position 146; Thr at position 156; Met at position 160; Arg at position 166; or His at position 177.

Such polypeptide can comprise or exhibit lipase activity or the ability to degrade geranyl butyrate, neryl butyrate, or both neryl and geranyl butyrate. The polypeptide can also exhibit enantioselectivity for geranyl butyrate over neryl butyrate. A polypeptide exhibiting enantioselectivity for geranyl butyrate over neryl butyrate can comprise one or more of: Arg at position 22; Gly at position 33; Ser or Lys at position 41; Arg at position 43; Ser at position 107; Lys at position 141; Lys at position 146; Met at position 160; or His at position 177, or can comprise one or more of: Arg at position 43; or Ser at position 107.

Such polypeptide can alternatively comprise or exhibit enantioselectivity for neryl butyrate over geranyl butyrate. Such polypeptide can comprise one or more of: Ser at position 17; Arg at position 22; Pro at position 31; Gly at position 33; Ser or Lys at position 41; Lys at position 141; Lys at position 146; Met at position 160; Arg at position 166; or His at position 177, or, can comprise one or more of: Ser at position 17; Pro at position 31; or Arg at position 166.

In another aspect, the invention can comprise an isolated or recombinant polypeptide comprising a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 55, 61, 64, 65, 67, 68, 70, or 72. Alternatively, such polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 55, e.g., the polypeptide can comprise a sequence selected from SEQ ID NO: 55, 58-62, 75-78, 80-88, or 94-108 (or the mature region thereof).

Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 61, which polypeptide, e.g., can comprise a sequence selected from SEQ ID NO: 55, 57-62, 75-78, 80-90, or 93-108.

5 Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 64, which polypeptide, e.g., can comprise a sequence selected from SEQ ID NO: 64, 71, or 72 (or the mature region thereof).

Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 65, which polypeptide, e.g., can comprise a sequence selected from SEQ ID NO: 65, 66, or 73 (or a mature region thereof).

10 Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 67, which polypeptide, e.g., can comprise the sequence SEQ ID NO: 67 (or the mature region thereof). Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 68, which polypeptide, e.g., can comprise a sequence selected  
15 from SEQ ID NO: 68 or 101 (or the mature region thereof). Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 70, which polypeptide, e.g., can comprise a sequence selected from SEQ ID NO: 63, 68-70, 82-83, 85-86, 96, or 101-102 (or the mature region thereof).

Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid  
20 sequence identity to the mature region of SEQ ID NO: 72, which polypeptide, e.g., can comprise a sequence selected from SEQ ID NO: 64, 71, or 72 (or a mature region thereof).

In another aspect, the invention can comprise an isolated or recombinant polypeptide comprising a sequence having at least 85% amino acid sequence identity to the mature region of SEQ ID NO: 74, which polypeptide, e.g., can comprise a sequence selected  
25 from SEQ ID NO: 63, 71-72, 74, or 79 (or a mature region thereof).

In yet another aspect, the invention can comprise an isolated or recombinant polypeptide comprising a sequence having at least 99% amino acid sequence identity to the mature region of SEQ ID NO: 56.

In other aspects, such isolated or recombinant polypeptide comprises an amino acid sequence of any one of SEQ ID NO: 55 through SEQ ID NO: 108 over a comparison window of at least 45 contiguous amino acids.

In some embodiments, the invention comprises an isolated or recombinant polypeptide that is at least 45 contiguous amino acid residues of a polypeptide encoded by a coding polynucleotide sequence wherein the polynucleotide sequence is selected from: a polynucleotide sequence from any of SEQ ID NO: 1 to SEQ ID NO: 54, a polynucleotide sequence that encodes a polypeptide selected from any of SEQ ID NO: 55 through SEQ ID NO: 108; or a polynucleotide sequence that hybridizes under stringent conditions over substantially the entire length of one of the above polynucleotide sequences or which hybridizes to a subsequence comprising at least about 100 nucleic acids, provided that the polynucleotide does not correspond to GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

Additionally, the invention provides such isolated or recombinant polypeptide wherein the polypeptide exhibits enantioselectivity for either a *cis* form enantiomer or a *trans* form enantiomer of a substrate and optionally wherein such enantioselectivity is represented by an enantiomeric ratio of at least 2 or more, at least 5 or more, at least 10 or more, at least 50 or more, or at least 100 or more.

In one embodiment, the invention, provides isolated or recombinant polypeptides encoded by a nucleic acid selected from any of the following: a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54 (or a complementary sequence thereof); a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108 (or a complementary polynucleotide sequence thereof); a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the whole length of any of the previously described polynucleotides, or which hybridizes to a

subsequence of the same comprising at least 100 residues wherein the polynucleotide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, 5 AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108; a polynucleotide sequence which comprises all, or a fragment of, any of the above described polynucleotides and which encodes a polypeptide comprising 10 lipase activity; or a polynucleotide sequence encoding a polypeptide which comprises an amino acid sequence that is substantially identical over at least 45 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108 wherein the polynucleotide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, 15 AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. Additionally, such polynucleotide as is produced by mutating 20 or recombining one or more of the above described polynucleotide sequences, is provided. The invention also provides an isolated or recombinant polypeptide as described above which comprises an amino acid sequence of any of SEQ ID NO: 55 to SEQ ID NO: 108.

In other aspects, the invention includes, isolated or recombinant polypeptides (as described above) which can optionally exhibit: lipase activity (e.g., with respect to 25 tributyrin, with respect to tributyrin in DMF, with respect to tributyrin after heat treatment (i.e., after the polypeptide has been heat treated); or enantioselective lipase activity (e.g., with respect to neryl-butyrate or geranyl-butyrate). Optionally, such polypeptides can comprise lipase activity against novel substrates (i.e., substrates upon which typical wild-type lipases do not act) such as, e.g., methyl esters, pentadecanolide, or oxacyclotridecan. Additionally, 30 such polypeptides optionally are substantially identical over at least 45, at least 50, at least

75, at least 100, at least 125, at least 150, at least 175, or at least 200 contiguous amino acids of any of the above described polypeptides with the proviso that the sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, 5 AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. Alternatively, such polypeptide is substantially identical over at least 180, at least 10 212, at least 213, or at least 215 contiguous amino acid residues of an above described polypeptide, again with the proviso that the sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, 15 BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

In various embodiments, the above described polypeptides further comprise 20 one or more of: a leader sequence, a precursor polypeptide, a secretion signal or a localization signal, an epitope tag, a fusion protein comprising one or more additional amino acid sequences, a polypeptide purification subsequence (e.g., an epitope tag, a FLAG tag, a polyhistidine sequence, a GST fusion), an N-terminus methionine residue, or a modified 25 amino acid (e.g., a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety or to an organic derivatizing agent).

Other aspects of the invention include, a composition of one or more modified amino acid polypeptide and a pharmaceutically acceptable excipient and/or a composition comprising one or more polypeptide of the invention with a surfactant (or with another 30 component of a cleaning solution such as a builder, a polymer, a bleach system, a structurant,



a pH adjuster, a humectant, or a neutral inorganic salt) or a pharmaceutically acceptable excipient.

Additionally, a polypeptide which comprises a unique subsequence selected from SEQ ID NO: 55 through SEQ ID NO: 108 which is unique as compared to a polypeptide sequence corresponding to an amino acid sequence (or which is encoded by a nucleic acid sequence) corresponding to any of GenBank accession numbers I16WA, I16WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108 is provided. Other aspects include a polypeptide which is specifically bound by a polyclonal antisera raised against at least one antigen comprising at least one amino acid sequence from SEQ ID NO: 55 to SEQ ID NO: 108 (or a fragment thereof) where the antisera is subtracted with a polypeptide corresponding to an amino acid sequence (or which is encoded by a nucleic acid sequence) corresponding to any of the above listed GenBank accession numbers.

In other aspects the invention includes an antibody or antisera produced by administering a polypeptide of the invention to a mammal and wherein the antibody or antisera specifically binds at least one antigen which comprises a polypeptide sequence (or fragment thereof) from SEQ ID NO: 55 to SEQ ID NO: 108 and which antibody or antisera does not specifically bind to a polypeptide encoded by a nucleic acid corresponding to, or an amino acid sequence corresponding to one or more of the above listed GenBank accession numbers.

In yet other aspects, the invention includes an antibody or antisera that specifically binds a polypeptide comprising an amino acid sequence (or fragment thereof) from SEQ ID NO: 55 to SEQ ID NO: 108 and which antibody or antisera does not specifically bind to a peptide encoded by a nucleic acid corresponding to, or an amino acid sequence corresponding to, one or more of the above listed GenBank accession numbers.

The invention also includes a nucleic acid comprising a sequence selected from: a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54 (or a complementary sequence thereof; a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108 (or a complementary sequence thereof); a  
5 polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of such sequences or which hybridizes to a subsequence thereof of at least 100 residues, provided that the polynucleotide sequence does not correspond to or encode any of GenBank accession numbers 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217,  
10 AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108; and a polynucleotide sequence comprising all or a fragment of any of the previous  
15 polynucleotides and which comprises lipase activity and, again, which does not correspond to or encode of the above listed GenBank accession numbers.

Other embodiments of the invention can comprise a nucleic acid which comprises a sequence which encodes a polypeptide having an amino acid sequence that is substantially identical over at least 45, at least 50, at least 75, at least 100, at least 125, at  
20 least 150, at least 175, or at least 200 contiguous amino acid residues of any of SEQ ID NO: 55 to SEQ ID NO: 108, and, again, which does not correspond to or encode of the above listed GenBank accession numbers. Additionally, the invention provides nucleic acid which comprises a sequence encoding a polypeptide having a sequence that is substantially identical over at least 180, at least 212, at least 213, or at least 215 contiguous amino acid residues of  
25 any of SEQ ID NO: 55 to SEQ ID NO: 108, and, which does not correspond to or encode of the above listed GenBank accession numbers.

Furthermore, the invention optionally provides such nucleic acids wherein the encoded polypeptide can optionally exhibit: lipase activity (e.g., against tributyrin, against tributyrin in DMF (dimethyl formamide), or against tributyrin after being heat treated (i.e.,  
30 after the polypeptide has been heat treated); enantioselective lipase activity (e.g., against

neryl-butyrate and/or geranyl-butyrate). Optionally, such nucleic acids can encode polypeptides which comprise lipase activity against novel substrates (i.e., substrates upon which typical wild-type lipases do not act) such as, e.g., methyl esters, pentadecanolide, or oxacyclotridecan. The invention also includes nucleic acids that comprise polynucleotide sequences encoding polypeptides comprising lipase activity and which are produced by mutating or recombining one or more polynucleotide sequence as described above (and which optionally comprises lipase activity) and/or an enantioselective lipase activity, and which do not correspond to or encode GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. The invention additionally provides any of the above described nucleic acids wherein the encoded polypeptide comprises: a leader sequence; a precursor peptide, an epitope tag sequence; or a fusion protein comprising one or more additional nucleic acid.

A composition comprising two or more nucleic acids of the invention, as well as such compositions that comprise a library (e.g., of at least about 2, 5, 10, 50, or more nucleic acids) is also a feature of the invention. Such compositions are optionally produced by cleaving of one or more nucleic acid (e.g., by mechanical, chemical or enzymatic (e.g., a restriction endonuclease, an RNase, a DNase, etc.) means) of any of the above described nucleic acids. Compositions produced by incubating one or more of any of the above described polynucleotides in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase (e.g., a thermostable polymerase) are also aspects of the current invention. Additionally, the invention provides a cell comprising at least one nucleic acid as described above (or a cleaved or amplified fragment or product thereof), which cell optionally expresses a polypeptide encoded by the nucleic acid. Vectors and/or expression vectors (e.g., plasmids, cosmids, phages, viruses, virus fragments, etc.) comprising any nucleic acid of the invention, as well as any cell transduced by such vectors are also provided. Compositions comprising any nucleic acid of the invention and a surfactant (or with another component of

a cleaning solution such as a builder, a polymer, a bleach system, a structurant, a pH adjuster, a humectant, or a neutral inorganic salt) and/or compositions comprising an excipient (optionally a pharmaceutically acceptable excipient) are also provided in the invention.

In one aspect, the invention provides a nucleic acid which comprises a unique  
5 subsequence selected from SEQ ID NO:1 to SEQ ID NO:54. The unique subsequence is unique as compared to a nucleic acid corresponding to any of the sequences represented, e.g., by GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652,  
10 CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108, or related sequences present in GenBank as of the filing of this application. Additionally, a target nucleic acid which hybridizes under stringent conditions to a unique coding oligonucleotide which encodes a  
15 unique subsequence in a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108, wherein the unique subsequence is unique as compared to an amino acid sequence or to a polypeptide encode by a nucleic acid sequence corresponding to any of the above GenBank accession numbers is also provided in the invention. Furthermore, in some embodiments the stringent conditions are selected such that a perfectly complementary oligonucleotide to the  
20 coding oligonucleotide hybridizes to the coding oligonucleotide with at least a 5x higher signal to noise ratio than for hybridization of the perfectly complementary oligonucleotide to a control nucleic acid corresponding to any of the above GenBank accession numbers and wherein the target nucleic acid hybridizes to the unique coding oligonucleotide with at least about a 2x higher signal to noise ratio as compared to hybridization of the control nucleic  
25 acid to the coding oligonucleotide.

In some embodiments, the current invention provides a database of one or more character strings corresponding to sequences selected from SEQ ID NO: 1 to SEQ ID NO: 108. Such database optionally comprises one or more character string recorded in a computer readable medium (e.g., internal or external to a computer). The invention also  
30 provides: a method for manipulating a sequence record in a computer system by reading a

character string (optionally selected by a user, e.g., from a database or inputted by the user into the computer system) corresponding to a sequence selected from SEQ ID NO: 1 to SEQ ID NO: 108 (or a subsequence thereof); performing an operation on the character string; and returning a result of the operation (optionally comprising transmitting the selected character string to an output device). The operations performed in such computer system optionally comprise any of the following: a local sequence comparison, a sequence alignment, a sequence identity or similarity search, a structural similarity search, a sequence identity or similarity determination, a structure determination, a nucleic acid motif determination, an amino acid motif determination, a hypothetical translation, a determination of a restriction map, a sequence recombination, or a BLAST determination. In some aspects the method can comprise: aligning the selected character string with one or more additional character strings corresponding to a polynucleotide or polypeptide sequence; translating one or more character strings from SEQ ID NO: 1 to SEQ ID NO: 54 into a character string corresponding to an amino acid sequence or translating a character string selected from SEQ ID NO: 55 to SEQ ID NO: 108, into a character string corresponding to a polynucleotide sequence; determining sequence identity or similarity between the selected character string and one or more additional character strings by evaluating codon usage (optionally determining optimal codon usage); and obtaining the result of the operation on a user output device (e.g., optionally selected from a display monitor, a printer, and an audio output). The method also comprises transmitting the character string to a device (e.g., an oligonucleotide synthesizer or peptide synthesizer) capable of producing a physical embodiment of the character string (e.g., a physical embodiment comprising a nucleic acid or polypeptide or peptide corresponding to a character string or a sub-portion thereof)

In some embodiments the invention provides methods of producing modified or recombinant nucleic acids comprising mutating or recombining (including through recursive recombination) a nucleic acid of the invention (or a fragment thereof), as well as the modified or recombinant nucleic acids that are produced by such method. Optionally, the one or more additional nucleic acid encodes a polypeptide comprising lipase activity and/or enantioselective lipase activity (or an amino acid subsequence or fragment thereof). The recombination (e.g., recursive recombination) is optionally done *in vitro* or *in vivo* and

optionally produces at least one library of recombinant nucleic acids, which comprises at least one polypeptide comprising lipase activity and/or enantioselective lipase activity (or a homologue thereof). Both the nucleic acid library produced and a population of cells comprising the library are provided by the invention, as are the modified or recombinant nucleic acids produced by the mutation/recombination (and cells which comprise such nucleic acids). In some aspects, the invention also provides a method of producing a polypeptide by introducing a nucleic acid of the invention (or a fragment thereof), which is operably linked to a regulatory sequence capable of directing expression of such nucleic acid, into a population of cells and then expressing the polypeptide. The polypeptide produced from such method is also part of the current invention. Such method optionally includes isolating the polypeptide from the cells and optionally includes expressing the polypeptide by culturing the population in a nutrient medium under conditions where the regulatory sequence directs expression of the polypeptide (again, wherein the polypeptide is optionally isolated or recovered from the cells and/or from the nutrient media (such culturing is optionally done in a bulk fermentation vessel)). The cells used in such methods are optionally bacterial, eukaryotic (e.g., fungal cells, yeast cells, plant cells, insect cells, or mammalian cells (e.g., fertilized oocytes, embryonic stem cells, pluripotent stem cells, etc.)). If mammalian cells are utilized, a transgenic animal is optionally regenerated from the cells and the polypeptide is optionally recovered from the transgenic animal or from a by-product of the transgenic animal such as milk.

In other aspects, the current invention provides methods/compositions for a cleaning solution (e.g., detergent) comprising the lipase homologues. Additional components (e.g., surfactants, proteolytic enzymes, humectants, neutral inorganic salts, sudsing agent, fragrance, structurants, etc.) can be included, individually, or multiply, in such compositions.

In yet other aspects, the current invention provides methods to therapeutically or prophylactically treat a gastrointestinal lipid related condition/disease/disorder by hydrolyzing a lipid through expressing a polypeptide in a target cell or contacting a target cell with an effective amount of polypeptide of the invention (or a fragment thereof) such target cell optionally is in culture or is within a subject to be treated. The current invention also provides a method of therapeutic or prophylactic treatment of a gastrointestinal lipid

related condition/disease/disorder in a subject wherein the subject is administered a polypeptide of the invention in an amount effective to treat the condition/disease/disorder, including wherein the subject is a mammal (e.g., a human), and wherein the polypeptide is administered in vivo, in vitro, or ex vivo (or a combination of such) to one or more cells of the subject. Such polypeptides include compositions of the polypeptide and a pharmaceutically acceptable excipient, which is administered to a subject in an amount effective to treat a gastrointestinal lipid related condition/disease/disorder (e.g., cystic fibrosis, celiac disease, Crohn's disease, indigestion, and obesity)

Another provision of the invention is a method of hydrolyzing a lipid to therapeutically or prophylactically treat a gastrointestinal lipid related condition/disease/disorder by introducing into a target cell a nucleic acid of the invention, or a fragment thereof, which is operably linked to a regulatory sequence active in the target cell such that introduction of the polynucleotide results in expression of the nucleic acid in an amount sufficient to hydrolyze the lipid. Such method optionally comprises directly administering the nucleic acid to a subject in an amount sufficient to introduce the nucleic acid into one or more cells. The subject optionally comprises a mammal (or a human) and the nucleic acid optionally comprises a vector. Yet another provision of the invention is a method of therapeutically or prophylactically treating a gastrointestinal lipid related condition/disease/disorder by expressing in a target cell (or contacting a target cell with an effective amount of) a polynucleotide of the invention, or a fragment thereof, or of a polypeptide encoded thereby (or a fragment thereof). Such method can include wherein the target cell is in culture or wherein the target cell is within a subject. Additionally, the invention provides a method of therapeutically or prophylactically treating a gastrointestinal lipid related condition/disease/disorder in a subject by administering to the subject a polynucleotide of the invention (or a fragment thereof) or a polypeptide encoded thereby (or a fragment thereof) in an amount effective to treat the gastrointestinal lipid related condition/disease/disorder. Such method comprises optional embodiments wherein the subject is a mammal or a human and wherein the polynucleotide and/or polypeptide is administered in vivo, in vitro, or ex vivo (or a combination of such) to one or more cells of the subject and wherein a composition of the polynucleotide and/or polypeptide and a

pharmaceutically acceptable excipient is administered to the subject in an amount effective to treat the gastrointestinal lipid related condition/disease/disorder (e.g., cystic fibrosis, celiac disease, Crohn's disease, indigestion, or obesity).

#### BRIEF DESCRIPTION OF THE FIGURES

- 5 Figure 1: Enantiomer Selectivity of Exemplary Lipase Homologues  
Figure 2: Enantiomeric Ratio for Exemplary Lipase Homologues.  
Figure 3a-3i: Alignment of Exemplary Novel Lipase Polynucleotides (SEQ  
ID NO: 1-20).  
Figure 4a-4h: Alignment of Exemplary Novel Lipase Polynucleotides  
10 (SEQ ID NO: 21-54).  
Figure 5a-5c: Alignment of Exemplary Novel Lipase Polypeptides (SEQ ID  
NO: 55-74).  
Figure 6a-6c: Alignment of Exemplary Novel Lipase Polypeptides (SEQ  
ID NO: 75-108).

#### 15 DETAILED DESCRIPTION OF THE INVENTION

##### DEFINITIONS

Unless otherwise defined herein or below in the remainder of the specification, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the present invention  
20 belongs.

A "polynucleotide sequence" is a nucleic acid (which is a polymer of nucleotides (A,C,T,U,G, etc. or naturally occurring nucleotide analogues, artificial nucleotide analogues, etc.) or a character string representing a nucleic acid, depending on context. Either the given nucleic acid or the complementary nucleic acid can be determined from any  
25 specified polynucleotide sequence.

Similarly, an "amino acid sequence" is a polymer of amino acids (a protein, polypeptide, etc.) or a character string representing an amino acid polymer, depending on context.



A "subsequence" or "fragment" is any portion of an entire sequence, up to and including the complete sequence.

"Substantially an entire length of a polynucleotide or amino acid sequence" refers to at least about 50%, at least about 60%, generally at least about 70%, generally at least about 80%, or typically at least about 90%, 95%, 96%, 97%, 98%, or 99% or more of a length of an amino acid sequence or nucleic acid sequence.

Numbering of a given amino acid or nucleotide polymer "corresponds to numbering" of a selected amino acid polymer or nucleic acid when the position of any given polymer component (amino acid residue, incorporated nucleotide, etc.) is designated by reference to the same residue position in the selected amino acid or nucleotide, rather than by the actual position of the component in the given polymer.

"Naturally occurring," as applied to an object, refers to the fact that the object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism, including viruses, that can be isolated from a source in nature and which has not been intentionally modified by humankind in the laboratory is naturally occurring. In one aspect, a "naturally occurring" nucleic acid (e.g., DNA or RNA) molecule is a nucleic acid molecule that exists in the same state as it exists in nature; that is, the nucleic acid molecule is not isolated, recombinant, or cloned.

A nucleic acid, protein, peptide, polypeptide, or other component is "isolated" when it is partially or completely separated from components with which it is normally associated (such as, other peptides, polypeptides, proteins (including complexes, e.g., polymerases and ribosomes which may accompany a native sequence), nucleic acids, cells, synthetic reagents, cellular contaminants, cellular components, etc.), e.g., such as from other components with which it is normally associated in the cell from which it was originally derived. A nucleic acid, polypeptide, or other component is substantially pure when it is partially or completely recovered or separated from other components of its natural environment such that it is the predominant species present in a composition, mixture, or collection of components (i.e., on a molar basis it is more abundant than any other individual species in the composition). In preferred embodiments, the preparation consists of more than 70%, typically more than 80%, or preferably more than 90% of the isolated species.

In one aspect, a "substantially pure" or "isolated" nucleic acid (e.g., RNA or DNA), polypeptide, protein, or composition also means wherein the object species (e.g., nucleic acid or polypeptide) comprises at least about 50, 60, or 70 percent by weight (on a molar basis) of all macromolecular species present. A substantially pure or isolated composition can also comprise at least about 80, 90, 95, 96, 97, 98, or 99 or more percent by weight of all macromolecular species present in the composition. An isolated object species can also be purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of derivatives of a single macromolecular species.

The term "isolated nucleic acid" can also refer to a nucleic acid (e.g., DNA or RNA) that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (i.e., one at the 5' and one at the 3' end) in the naturally occurring genome of the organism from which the nucleic acid of the invention is derived. Thus, this term includes, e.g., a cDNA or a genomic DNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease treatment, whether such cDNA or genomic DNA fragment is incorporated into a vector, integrated into the genome of the same or a different species than the organism, including, e.g., a virus, from which it was originally derived, linked to an additional coding sequence to form a hybrid gene encoding a chimeric polypeptide, or independent of any other DNA sequences. The DNA may be double-stranded or single-stranded, sense or antisense.

A nucleic acid or polypeptide is "recombinant" when it is artificial or engineered, or derived from an artificial or engineered protein or nucleic acid. The term "recombinant" when used with reference e.g., to a cell, nucleotide, vector, or polypeptide typically indicates that the cell, nucleotide, or vector has been modified by the introduction of a heterologous (or foreign) nucleic acid or the alteration of a native nucleic acid, or that the polypeptide has been modified by the introduction of a heterologous amino acid, or that the cell is derived from a cell so modified. Recombinant cells express nucleic acid sequences (e.g., genes) that are not found in the native (non-recombinant) form of the cell or express native nucleic acid sequences (e.g., genes) that would be abnormally expressed, under-expressed, or not expressed at all.

The term "recombinant nucleic acid" (e.g., DNA or RNA) molecule means, for example, a nucleotide sequence that is not naturally occurring or is made by the combination (for example, artificial combination) of at least two segments of sequence that are not typically included together, not typically associated with one another, or are otherwise typically separated from one another. A recombinant nucleic acid can comprise a nucleic acid molecule formed by the joining together or combination of nucleic acid segments from different sources and/or artificially synthesized. The term "recombinantly produced" refers to an artificial combination usually accomplished by either chemical synthesis means, recursive sequence recombination of nucleic acid segments or other diversity generation methods of nucleotides, or manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques known to those of ordinary skill in the art. "Recombinantly expressed" typically refers to techniques for the production of a recombinant nucleic acid in vitro and transfer of the recombinant nucleic acid into cells in vivo, in vitro, or ex vivo where it may be expressed or propagated. A "recombinant polypeptide" or "recombinant protein" usually refers to polypeptide or protein, respectively, that results from a cloned or recombinant gene or nucleic acid.

A "vector" is a composition for facilitating cell transduction by a selected nucleic acid, or expression of the nucleic acid in the cell. Vectors include, e.g., plasmids, cosmids, viruses, YACs, bacteria, poly-lysine, etc. An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specific nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. The expression vector typically includes a nucleic acid to be transcribed operably linked to a promoter.

The term "homology" generally refers to the degree of similarity between two or more structures. The term "homologous sequences" refers to regions in macromolecules that have a similar order of monomers. When used in relation to nucleic acid sequences, the term "homology" refers to the degree of similarity between two or more nucleic acid sequences (e.g., genes) or fragments thereof. Typically, the degree of similarity between two or more nucleic acid sequences refers to the degree of similarity of the composition, order, or arrangement of two or more nucleotide bases (or other genotypic feature) of the two or more

nucleic acid sequences. The term "homologous nucleic acids" generally refers to nucleic acids comprising nucleotide sequences having a degree of similarity in nucleotide base composition, arrangement, or order. The two or more nucleic acids may be of the same or different species or group. The term "percent homology" when used in relation to nucleic acid sequences, refers generally to a percent degree of similarity between the nucleotide sequences of two or more nucleic acids.

When used in relation to polypeptide (or protein) sequences, the term "homology" refers to the degree of similarity between two or more polypeptide (or protein) sequences (e.g., genes) or fragments thereof. Typically, the degree of similarity between two or more polypeptide (or protein) sequences refers to the degree of similarity of the composition, order, or arrangement of two or more amino acids of the two or more polypeptides (or proteins). The two or more polypeptides (or proteins) may be of the same or different species or group. The term "percent homology" when used in relation to polypeptide (or protein) sequences, refers generally to a percent degree of similarity between the amino acid sequences of two or more polypeptide (or protein) sequences. The term "homologous polypeptides" or "homologous proteins" generally refers to polypeptides or proteins, respectively, that have amino acid sequences and functions that are similar. Such homologous polypeptides or proteins may be related by having amino acid sequences and functions that are similar, but are derived from, or evolved from, different or the same species using the techniques described herein.

The term "subject" as used herein includes, but is not limited to, an organism; mammal, including, e.g., human, non-human primate (e.g., monkey), mouse, pig, cow, goat, rabbit, rat, guinea pig, hamster, horse, monkey, sheep, or other non-human mammal; a non-mammal, including, e.g., a non-mammalian vertebrate, such as a bird (e.g., chicken or duck) or a fish; and a non-mammalian invertebrate.

The term "pharmaceutical composition" means a composition suitable for pharmaceutical use in a subject, including an animal or human. A pharmaceutical composition generally comprises an effective amount of an active agent and a pharmaceutically acceptable carrier.

The term "effective amount" means a dosage or amount sufficient to produce a desired result. The desired result may comprise an objective or subjective improvement in the recipient which receives the dosage or amount.

5 A "prophylactic treatment" is a treatment administered to a subject who does not display signs or symptoms of a disease, pathology, or medical disorder, or displays only early signs or symptoms of a disease, pathology, or disorder, such that treatment is administered for the purpose of diminishing, preventing, or decreasing the risk of developing the disease, pathology, or medical disorder. A prophylactic treatment functions as a preventative treatment against a disease or disorder. A "prophylactic activity" is an activity  
10 of an agent, such as a nucleic acid, vector, gene, polypeptide, protein, substance, or composition thereof that, when administered to a subject who does not display signs or symptoms of pathology, disease or disorder, or who displays only early signs or symptoms of pathology, disease, or disorder, diminishes, prevents, or decreases the risk of the subject developing a pathology, disease, or disorder. A "prophylactically useful" agent or compound  
15 (e.g., nucleic acid or polypeptide) refers to an agent or compound that is useful in diminishing, preventing, treating, or decreasing development of pathology, disease or disorder.

A "therapeutic treatment" is a treatment administered to a subject who displays symptoms or signs of pathology, disease, or disorder, in which treatment is  
20 administered to such subject for the purpose of diminishing or eliminating those signs or symptoms of pathology, disease, or disorder. A "therapeutic activity" is an activity of an agent, such as a nucleic acid, vector, gene, polypeptide, protein, substance, or composition thereof, that eliminates or diminishes signs or symptoms of pathology, disease or disorder, when administered to a subject suffering from such signs or symptoms. A "therapeutically  
25 useful" agent or compound (e.g., nucleic acid or polypeptide) indicates that an agent or compound is useful in diminishing, treating, or eliminating such signs or symptoms of a pathology, disease or disorder.

The term "gene" broadly refers to any segment of DNA associated with a biological function. Genes include coding sequences and/or regulatory sequences required  
30 for their expression. Genes also include non-expressed DNA nucleic acid segments that, e.g.,

form recognition sequences for other proteins (e.g., promoter, enhancer, or other regulatory regions).

Generally, the nomenclature used herein, and the laboratory procedures in cell culture, molecular genetics, molecular biology, nucleic acid chemistry, and protein chemistry described below, are those well known and commonly employed by those of ordinary skill in the art. Standard techniques, such as described in Sambrook et al., *Molecular Cloning - A Laboratory Manual* (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 (hereinafter "Sambrook") and *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (supplemented through 2000) (hereinafter "Ausubel"), are used for recombinant nucleic acid methods, nucleic acid synthesis, cell culture methods, and transgene incorporation, e.g., electroporation, injection, and lipofection. Generally, oligonucleotide synthesis and purification steps are performed according to specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references which are provided throughout this document. The procedures herein are believed to be well known to those of ordinary skill in the art and are provided for the convenience of the reader.

As used herein, an "antibody" refers to a protein comprising one or more polypeptides substantially or partially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. A typical immunoglobulin (e.g., antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" chain (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these light and heavy chains, respectively. Antibodies exist as intact immunoglobulins or

as a number of well characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)<sub>2</sub>, a dimer of Fab which itself is a light chain joined to VH-CH1 by a disulfide bond. The F(ab)<sub>2</sub> may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the (Fab)<sub>2</sub> dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (*see, Fundamental Immunology*, W.E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such Fab' fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies. Antibodies include single chain antibodies, including single chain Fv (sFv) antibodies in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide.

The term "lipase activity" refers herein to the ability of a lipase enzyme to hydrolyze a lipid, oil, or fat molecule, detected by, for example, any of the lipase activity assays described herein or known to those having ordinary skill in the art (*see, e.g., EXAMPLE I and the references cited therein*).

"Enantioselective lipase activity" refers herein to the ability of a lipase enzyme to preferentially hydrolyze a specific enantiomer of a lipid, oil, or fat molecule, detected by, for example, any of the enantioselective lipase activity assays described herein (*see, e.g., EXAMPLE II and the references cited therein*).

A "mature region" as used herein refers to the mature coding region of a polypeptide, i.e., it does not include the signal peptide coding region. For example, Figures 3 and 5 depict the mature coding regions of exemplary lipases of the current invention.

An "equivalent amino acid position" is defined herein as an amino acid position of a test polypeptide which aligns with an amino acid position of SEQ ID NO:75 using an alignment algorithm as described herein. The equivalent amino acid position of the test polypeptide need not be the same as the linear amino acid sequence position of the test

polypeptide. As an example, amino acid number 2 of the polypeptide SEQ ID NO:75 is considered to be the equivalent amino acid position to amino acid number 35 of the polypeptide SEQ ID NO:55 and to amino acid number 38 of SEQ ID NO:65, since amino acid number 2 of SEQ ID NO:75 aligns with amino acid number 35 of SEQ ID NO:55 and  
5 with amino acid number 38 of SEQ ID NO:65 using an alignment algorithm described herein, e.g., the CLUSTALW alignment program using default parameters. Therefore, "amino acid position 2 or an equivalent position to that of SEQ ID NO:75" is meant to correspond, e.g., to amino acid 35 of SEQ ID NO:55, amino acid 38 of SEQ ID NO:65, etc.

A variety of additional terms are defined or otherwise characterized herein.

## 10 POLYNUCLEOTIDES

### Novel lipase Sequences

The invention provides isolated or recombinant lipase polypeptides and homologues thereof (optionally collectively referred to as lipase polypeptides), and isolated or recombinant polynucleotides encoding the polypeptides.

### 15 Novel Lipase Molecules and Lipase Variants

The present invention relates to the isolation of newly discovered lipase polynucleotides from different strains of *Bacillus* as well as creation of novel lipase polynucleotides. A number of *Bacillus* species (both known *Bacillus* species and un-typed *Bacillus* species) were screened to identify lipase activity while in colonies. Plate screens  
20 were used to identify those colonies expressing lipase activity. See, "EXAMPLE I" below, and, e.g., Dartois, V. et al., "Cloning, nucleotide sequence and expression in *Escherichia coli* of a lipase gene from *Bacillus subtilis* 168," Biochimica et Biophysica Acta 1131 (1992) 253-260 and references cited therein.

DNA from colonies which displayed lipase activity was used in PCR reactions  
25 with degenerate lipase primers designed to a known lipase gene from *Bacillus subtilis*. For reactions that did not readily produce amplified lipase genes, the DNA isolates were amplified using internal degenerative primers designed to anneal to more conserved regions, thus producing lipase gene fragments which were spliced into *B. subtilis* to generate chimeric full-length genes. The techniques used for amplification, etc. are well known to those of skill  
30 in the art and references teaching such are replete herein. The lipase genes discovered



through this process (SEQ ID NO: 1 through SEQ ID NO: 20) correspond to lipase homologues polypeptides shown in SEQ ID NO: 55 through SEQ ID NO: 74. Novel lipase polynucleotides were isolated from cultures of *B. pumilus*, *B. subtilis*, *B. megaterium*, *B. lentus*, *B. circulans*, *B. azotoformans*, *B. firmus*, and *B. badius* (see, SEQ ID NO: 1 through SEQ ID NO: 8 and SEQ ID NO: 55 through SEQ ID NO: 62) as well as from undetermined *Bacillus* species (see, SEQ ID NO: 9 through SEQ ID NO: 20 and SEQ ID NO: 63 through SEQ ID NO: 74). See, Figures 3 and 5.

The newly isolated *Bacillus* lipase polynucleotides were then recombined to create libraries of novel lipase homologues which were screened for lipase activity and enantioselectivity (see, "EXAMPLE I" and *infra*). A number of homologues were chosen for further analysis (i.e., the novel lipase homologues of the invention). Methods and protocols for generation of nucleic acid libraries and of nucleic acid recombination are well known to those of skill in the art and can be found in numerous references cited herein. The nucleic acids for both the discovered *Bacillus* lipases and the newly created lipases were cloned into *E. coli* expression vectors, transformed in to *E. coli*, and screened for lipase activity (see, below for screening).

Sequences of the newly created lipase polynucleotides (i.e., those created through recombination of the newly isolated lipase genes) are shown in SEQ ID NO: 21 through SEQ ID NO: 54 (with the corresponding amino acid sequences being SEQ ID NO: 75 through SEQ ID NO: 108). It should be noted that the nucleic acid sequences of the created lipase homologues (SEQ ID NO: 21 through SEQ ID NO: 54) are present in the sequence listing table herein with an introductory 5' 'T' and an ending 3' 'TGA,' used for, e.g., construction of vector attachment sites, etc. and which, in many embodiments of the invention, are optionally removed or are not present. See, Figures 4 and 6

The newly created lipase homologues of the invention (i.e., SEQ ID NO: 21 through SEQ ID NO: 54 and SEQ ID NO: 75 through SEQ ID NO: 108) were also examined for enantioselectivity. Enantioselectivity as used herein, refers to the preference of an enzyme (e.g., lipase) to selectively utilize one substrate enantiomer over another enantiomer. Enantiomers are stereoisomers that are non-superimposable mirror images of each other. For example, neryl-butyrate and geranyl-butyrate are enantiomers of one another. It will be

appreciated that while the screen was for enantioselectivity against neryl or geranyl butyrate, the novel lipase homologues herein optionally show lipase activity and/or enantioselective lipase activity against other substrates, e.g., neryl or geranyl acetate, other *cis/trans* lipids or lipid esters, etc.

5           While enantiomers have the same basic structure, they can vary in some specifics. For example the *cis/trans* enantiomers neryl- butyrate and geranyl- butyrate are used for different processes in the perfume/fragrance industry. Thus, enzymatic pathways that specifically produce one or the other (i.e., either neryl or geranyl butyrate) would be a welcome addition. Of course, myriad other enantiomers (both known and unknown) are also  
10 useful in numerous processes/applications and neryl/geranyl butyrate is only a non-limiting example of possible enantiomeric substrates for the lipase homologues of the invention.

          The present invention also provides enantioselective lipases. Enantioselectivity can be readily determined as described below by comparing the conversion of such substrate enantiomers. For example, enantioselectivity was detected by  
15 growing clones expressing lipases of the present invention on media containing neryl- butyrate and geranyl- butyrate. The neryl- butyrate and geranyl- butyrate created a hazy appearance in the media on which the library constituents were grown. If an individual colony of a library produced active lipase (either secreted lipase or lipase from lysed cells) that utilized the neryl and/or geranyl butyrate in the media, it would break it down and clear  
20 that area of the plate. In other words, the colonies containing active lipase (which could breakdown the neryl- butyrate and/or geranyl- butyrate) produced a clear ring or halo around the colony. Such colonies were isolated and further analyzed to check for enantioselectivity. The protocol followed corresponded to that found in "SCREENING FOR ENZYME  
STEREOSELECTIVITY UTILIZING MASS SPECTROMETRY," by Davis et al., Attorney  
25 Docket Number 02-109010US, USSN 60/278934 filed March 26, 2001. While all the sequences used to create the libraries (i.e., SEQ ID NO: 1 – 20 (nucleic acid) and SEQ ID NO: 55-74 (polypeptide)) displayed enantioselectivity for geranyl-butyrate, a number of the novel lipase homologues of the invention surprisingly display enantioselectivity for neryl- butyrate while other lipase homologue polypeptides displayed greater geranyl  
30 enantioselective lipase activity than the parental clones. See, Figures 1 and 2 which list the

enantioselectivity (i.e., either for geranyl butyrate or neryl butyrate) and selected enantiomeric ratio values for selected lipase homologues.

As described in USSN 60/278934, the phrase "enzyme stereoselectivity" refers to the preference for one substrate stereoisomer or *pseudo*-stereoisomer (if one form is labeled) over another or others in a chemical reaction catalyzed by an enzyme. When the stereoisomers are enantiomers, the phenomenon is referred to as "enzyme enantioselectivity" and is quantitatively expressed by the enantiomeric excess or the enantiomeric ratio. "Enantiomeric excess" refers to the absolute difference between the mole or weight fractions of major ( $F_{(+)}$ ) and minor ( $F_{(-)}$ ) enantiomers (i.e.,  $|F_{(+)} - F_{(-)}|$ ), where  $F_{(+)} + F_{(-)} = 1$ . The percent enantiomer excess is  $100 |F_{(+)} - F_{(-)}|$ . The enantiomeric ratio is determined by the following equation:

$$E = \frac{\ln [ 1 - c ( 1 + DE(p) ) ]}{\ln [ 1 - c ( 1 - DE(p) ) ]}$$

where  $c$  = the percent total substrate conversion (expressed as a decimal), and  $DE(p)$  is the diastereomeric excess (i.e., the percent product of isomer "1" less the percent product of isomer "2").

Employing the methods described herein and in USSN 60/278934, it was determined that polypeptide sequences SEQ ID NOS: 55 to 74 displayed enantioselectivity for geranyl butyrate *versus* neryl butyrate. As an example, an E (Enantiomeric ratio) value for an exemplary newly discovered lipase homologue has a geranyl enantiomer of about 2. See, Figure 2.

A number of novel lipase homologues of the invention displayed enantioselectivity for geranyl butyrate *versus* neryl butyrate greater than that of the parental sequences. For example, 2 exemplary homologues having a preference for the geranyl enantiomer have E values of at least about 3 or more.

Surprisingly, none of SEQ ID NO: 1-20 (SEQ NO ID 55-74 for corresponding polypeptides) displayed enantioselectivity for neryl butyrate, yet a number of the other lipases of the present invention did displayed enantioselectivity for neryl butyrate *versus* geranyl butyrate, with E values for the neryl enantiomer of at least about 1.4 up to about 2.2 for selected homologues. See, Figure 2.

### Novel Substitutions

Certain lipase homologues of the invention (e.g., SEQ ID NOS: 75 to 108) contain one or more of the following amino acid substitutions: Lys at position 1, Thr at position 14, Ser at position 17, Arg at position 22, Glu at position 26, Pro at position 31, Gly at position 33, Glu at position 34, Pro at position 35, Pro or Thr at position 37, Ser or Lys at position 41, Gly at position 42, Arg or Glu at position 43, Ala at position 61, Tyr at position 75, Gly at position 96, Ser at position 97, Thr at position 104, Ser at position 107, Ala at position 125, Gly at position 129, Val at position 134, Cys at position 138, Lys at position 141, Lys at position 146, Thr at position 156, Met at position 160, Arg at position 166, or His at position 177, which are not found in equivalent amino acid positions of related lipase sequences having GenBank Protein Accession Nos. AAA22574, CAB95850, CAB12664, BAA11406, CAA02196, CAA00273, CAB12064, BAA22231, and CAA00274. An equivalent amino acid position is defined *supra* as an amino acid position of a test polypeptide which aligns with an amino acid position of SEQ ID NO:75 (*see, supra*).

Preferred amino acid substitutions include those which are observed in a number of the lipase homologues of the invention which display enantioselectivity for geranyl butyrate *versus* neryl butyrate (e.g., having E values of at least about 3 for the geranyl enantiomer): Arg at position 22, Gly at position 33, Ser or Lys at position 41, Arg at position 43, Ser at position 107, Lys at position 141, Lys at position 146, Met at position 160, and His at position 177. More preferred substitutions include those which are observed only in lipase homologues of the invention which display enantioselectivity for geranyl enantiomer: Arg at position 43 and Ser at position 107.

Preferred amino acid substitutions also include those which are observed in a number of the lipase homologues of the invention which display enantioselectivity for neryl butyrate *versus* geranyl butyrate (e.g., having E values of at least about 1.4 for the neryl enantiomer): Ser at position 17, Arg at position 22, Pro at position 31, Gly at position 33, Ser or Lys at position 41, Lys at position 141, Lys at position 146, Met at position 160, Arg at position 166, or His at position 177. More preferred substitutions include those which are observed only in lipase homologues of the invention which display enantioselectivity for the neryl enantiomer: Ser at position 17, Pro at position 31, and Arg at position 166.

The nucleic acid sequences of the current invention (i.e., SEQ ID NO: 1 through SEQ ID NO: 54) can be recombined (or further recombined) in accordance with the methods described herein and expressed in, e.g., *E. coli* to generate additional lipase variants. Lipase activity can be screened for on, e.g., tributyrin and further parameters such as, e.g. 5 thermostability, lipase activity on novel substrates (i.e., on substrates on which known lipase variants do not have activity, etc.) can be selected for.

#### Making Polynucleotides

Polynucleotides and oligonucleotides of the invention can be prepared by standard solid-phase methods, according to known synthetic methods. Typically, fragments 10 of up to about 100 bases are individually synthesized, then joined (e.g., by enzymatic or chemical ligation methods, or polymerase mediated recombination methods) to form essentially any desired continuous sequence. For example, the polynucleotides and oligonucleotides of the invention can be prepared by chemical synthesis using, e.g., the classical phosphoramidite method described by Beaucage et al., (1981) Tetrahedron Letters 15 22:1859-69, or the method described by Matthes et al., (1984) EMBO J 3: 801-05., e.g., as is typically practiced in automated synthetic methods. According to the phosphoramidite method, oligonucleotides are synthesized, e.g., in an automatic DNA synthesizer, purified, annealed, ligated and cloned in appropriate vectors.

In addition, essentially any nucleic acid can be custom ordered from any of a 20 variety of commercial sources, such as The Midland Certified Reagent Company (mcrc@oligos.com), The Great American Gene Company (www.genco.com), ExpressGen Inc. (www.expressgen.com), Operon Technologies Inc. (Alameda, CA) and many others. Similarly, peptides and antibodies can be custom ordered from any of a variety of sources, such as PeptidoGenic (pkim@ccnet.com), HTI Bio-products, inc. (www.htibio.com), BMA 25 Biomedicals Ltd. (U.K.), Bio.Synthesis, Inc., and many others.

Certain polynucleotides of the invention may also be obtained by screening cDNA libraries (e.g., libraries generated by recombining homologous nucleic acids as in typical recursive recombination methods) using oligonucleotide probes which can hybridize to, or PCR-amplify, polynucleotides which encode the novel lipase polypeptides and 30 fragments of those polypeptides. Procedures for screening and isolating cDNA clones are

well-known to those of skill in the art. Such techniques are described in, for example, Sambrook et al. (1989) *supra*, and Ausubel FM et al. (1989; supplemented through 2000) *supra*.

As described in more detail herein, the polynucleotides of the invention  
5 include sequences which encode novel lipase homologues and sequences complementary to the coding sequences, and novel fragments of coding sequence and complements thereof. The polynucleotides can be in the form of RNA or in the form of DNA, and include mRNA, cRNA, synthetic RNA and DNA, and cDNA. The polynucleotides can be double-stranded or single-stranded, and if single-stranded, can be the coding strand or the non-coding (anti-  
10 sense, complementary) strand. The polynucleotides optionally include the coding sequence of a novel lipase homologue (i) in isolation, (ii) in combination with additional coding sequence, so as to encode, e.g., a fusion protein, a precursor protein, a protein comprising a leader sequence, or the like, (iii) in combination with non-coding sequences, such as introns (including artificial introns), control elements such as a promoter, a terminator element, or 5'  
15 and/or 3' untranslated regions effective for expression of the coding sequence in a suitable host, and/or (iv) in a vector or host environment in which the novel lipase coding sequence is a heterologous gene. Sequences can also be found in combination with typical compositional formulations of nucleic acids, including in the presence of carriers, buffers, adjuvants, excipients and the like.

20

#### Using Polynucleotides

The polynucleotides (and polypeptides) of the invention have a variety of uses including, but not limited to, for example: recombinant production (i.e., expression) of the recombinant lipase polypeptides of the invention for industrial and other uses (e.g., especially as components of cleaning solutions such as laundry detergents, dish detergents, industrial  
25 cleansers (e.g., for septic systems, grease traps, machinery parts, etc.)); as therapeutic and prophylactic agents in methods of in vivo and ex vivo treatment of a variety of diseases, disorders, and conditions; for use in in vitro methods, such as diagnostic and screening methods, to detect, diagnose, and treat a variety of diseases, disorders, and conditions (e.g., pancreatic disorders) in a variety of subjects (e.g., mammals); as immunogens; in gene  
30 therapy methods and DNA- or RNA-based delivery methods to deliver or administer in vivo,

ex vivo, or in vitro, biologically active polypeptides of the invention to a tissue, population of cells, organ, graft, bodily system of a subject (e.g., organ system, lymphatic system, blood system, etc.); as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural lipase coding nucleic acids);  
5 as substrates for further reactions, e.g., recursive recombination reactions, mutation reactions, or other diversity generation reactions to produce new and/or improved lipase homologues, and new lipase nucleic acids encoding such homologues, e.g., to evolve novel therapeutic, prophylactic, or industrial properties, and the like; for polymerase chain reactions (PCR) or cloning methods, e.g., including digestion or ligation reactions, to identify new and/or  
10 improved naturally-occurring or non-naturally occurring lipase nucleic acids and polypeptides encoded therefrom. Polynucleotides which encode a lipase homologue of the invention, or complements of the polynucleotides, are optionally administered to a cell to accomplish a therapeutically or prophylactically useful process or to express a therapeutically useful product in vivo, ex vivo, or in vitro.

15           The present invention provides an isolated or recombinant nucleic acid comprising a polynucleotide sequence selected from: a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54 (or a complementary polynucleotide sequence thereof); a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108 (or a complementary polynucleotide thereof); a polynucleotide sequence  
20 which hybridizes under highly stringent conditions over substantially the entire length of such polynucleotide sequences or which hybridizes to a subsequence thereof of at least 100 residues provided that the polynucleotide sequence does not correspond to or encode any of GenBank accession numbers 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840,  
25 AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108; and a polynucleotide sequence comprising all or a fragment of any of the previous polynucleotides and which comprises

lipase activity and which does not correspond to or encode any of the above GenBank accession numbers.

Other embodiments of the invention can comprise an isolated or recombinant nucleic acid which comprises a polynucleotide sequence which encodes a polypeptide having  
5 an amino acid sequence that is substantially identical over at least 45, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, or at least 200 contiguous amino acid residues of any of SEQ ID NO: 55 to SEQ ID NO: 108 provided that the polynucleotide sequence does not correspond to or encode any of GenBank accession numbers 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278,  
10 AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. Additionally, the invention provides an isolated or recombinant nucleic acid  
15 which comprises a polynucleotide sequence which encodes a polypeptide having an amino acid sequence that is substantially identical over at least 180, at least 212, at least 213, or at least 215 contiguous amino acid residues of any of SEQ ID NO: 55 to SEQ ID NO: 108, provided that the sequence does not correspond to or encode any of the GenBank accession numbers listed above.

20 Furthermore, the invention provides such nucleic acids as described wherein the encoded polypeptide comprises lipase activity (e.g., against tributyrin, against tributyrin in DMF (dimethyl formamide), against tributyrin after being heat treated (i.e., after the polypeptide has been heat treated); and/or comprises enantioselective lipase activity (e.g., against neryl- butyrate or geranyl- butyrate). Optionally, such nucleic acids as described can  
25 encode polypeptides which comprise lipase activity against novel substrates (i.e., substrates upon which typical wild-type lipases do not act) such as, e.g., methyl esters, pentadecanolide, or oxacyclotridecan. The invention also includes isolated or recombinant nucleic acids that comprise a polynucleotide sequence which encodes a polypeptide comprising lipase activity and which is produced by mutating or recombining one or more polynucleotide sequence as  
30 described above (and which optionally comprises lipase activity) providing that the sequence



does not correspond to or encode any of the GenBank accession sequences above. The invention additionally provides any of the above described nucleic acids wherein the encoded polypeptide comprises: a leader sequence; a precursor peptide, an epitope tag sequence; or a fusion protein comprising one or more additional nucleic acid.

5                   A composition comprising two or more nucleic acids as described above, as well as such compositions that comprise a library (e.g., of at least about 2, 5, 10, 50, or more nucleic acids) is also a feature of the invention. Such compositions are optionally produced by cleaving of one or more nucleic acid (e.g., by mechanical, chemical or enzymatic (e.g., a restriction endonuclease, an RNase, a DNase, etc.) means) of any of the above described  
10                   nucleic acids. Compositions produced by incubating one or more of any of the above described polynucleotides in the presence of deoxyribonucleotide triphosphates and a nucleic acid polymerase (e.g., a thermostable polymerase) are also provided in the current invention. Additionally, the invention provides a cell (which optionally expresses a polypeptide encoded by the nucleic acid) comprising at least one nucleic acid as described above (or a  
15                   cleaved or amplified fragment or product thereof). Vectors and/or expression vectors (e.g., plasmids, cosmids, phages, viruses, virus fragments, etc.) comprising any nucleic acid as described above, as well as any cell transduced by such vectors are also provided. Compositions comprising any nucleic acid as described above and an excipient (optionally a pharmaceutically acceptable excipient are also provided in the invention).

## 20                   EXPRESSION OF POLYPEPTIDES

                  In accordance with the present invention, polynucleotide sequences which encode novel lipase homologues (including mature lipase homologues), fragments of lipase proteins, related fusion proteins, or functional equivalents thereof, collectively referred to herein, e.g., as "lipase homologue polypeptides," "novel lipase polypeptides," or "lipase  
25                   polypeptides" are used in recombinant DNA molecules that direct the expression of the lipase homologue polypeptides in appropriate host cells. Due to the inherent degeneracy of the genetic code, other nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence are also used to clone and express the lipase homologues.

#### Modified Coding Sequences:

As will be understood by those of skill in the art, it can be advantageous to modify a coding sequence to enhance its expression in a particular host. The genetic code is redundant with 64 possible codons, but most organisms preferentially use a subset of these codons. The codons that are utilized most often in a species are called optimal codons, and those not utilized very often are classified as rare or low-usage codons (*see, e.g., Zhang SP et al. (1991) Gene 105:61-72*). Codons can be substituted to reflect the preferred codon usage of the host, a process called "codon optimization" or "controlling for species codon bias."

Optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host (*see also, Murray, E. et al. (1989) Nuc Acids Res 17:477-508*) can be prepared, for example, to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced from a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for *S. cerevisiae* and mammals are UAA and UGA respectively. The preferred stop codon for monocotyledonous plants is UGA, whereas insects and *E. coli* prefer to use UAA as the stop codon (*Dalphin ME et al. (1996) Nuc Acids Res 24: 216-218*).

The polynucleotide sequences of the present invention can be engineered in order to alter lipase homologue coding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, alterations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns or other conjugation patterns, to change codon preference, to introduce splice sites, to introduce or remove introns, etc.

#### Vectors, Promoters and Expression Systems.

The present invention also includes recombinant constructs comprising one or more of the nucleic acid sequences as broadly described above. The constructs comprise a vector, such as, a plasmid, a cosmid, a phage, a virus, a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), and the like, into which a nucleic acid sequence of the invention has been inserted, e.g., a polynucleotide encoding a lipase homologue, in a forward or reverse orientation. In a preferred aspect of this embodiment, the

construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

General texts which describe molecular biological techniques useful herein, including the use of vectors, promoters and many other relevant topics, include Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA (Berger); Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology, Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel"). Examples of techniques sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Q $\beta$ -replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) U.S. Patent No. 4,683,202; PCR Protocols A Guide to Methods and Applications (Innis et al. eds.) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) C&EN 36-47; The Journal Of NIH Research (1991) 3, 81-94; (Kwoh et al. (1989) Proc Natl Acad Sci USA 86, 1173; Guatelli et al. (1990) Proc Natl Acad Sci USA 87, 1874; Lomeli et al. (1989) J Clin Chem 35, 1826; Landegren et al., (1988) Science 241, 1077-1080; Van Brunt (1990) Biotechnology 8, 291-294; Wu and Wallace, (1989) Gene 4, 560; Barringer et al. (1990) Gene 89, 117, and Sooknanan and Malek (1995) Biotechnology 13: 563-564. Improved methods of cloning in vitro amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039. Improved methods of amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR expansion and sequencing using reverse transcriptase and a polymerase. See, Ausubel, Sambrook and Berger, *all supra*.

The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention by recombinant techniques. Host cells are genetically engineered (i.e., transduced, transformed or transfected) with the vectors of this invention, which can be, for example, a cloning vector or an expression vector. The vector can be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants, or amplifying the lipase homologue gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, e.g., Freshney (1994) *Culture of Animal Cells, a Manual of Basic Technique*, third edition, Wiley- Liss, New York and the references cited therein.

The lipase homologue proteins of the invention can also be produced in non-animal cells such as plants, yeast, fungi, bacteria and the like. In addition to Sambrook, Berger and Ausubel, details regarding cell culture can be found in Payne *et al.* (1992) *Plant Cell and Tissue Culture in Liquid Systems* John Wiley & Sons, Inc. New York, NY; Gamborg and Phillips (eds.) (1995) *Plant Cell, Tissue and Organ Culture; Fundamental Methods* Springer Lab Manual, Springer-Verlag (Berlin Heidelberg New York) and Atlas and Parks (eds.) *The Handbook of Microbiological Media* (1993) CRC Press, Boca Raton, FL.

The polynucleotides of the present invention may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, pseudorabies, adeno-associated virus, retroviruses and many others. Any vector that transducers genetic material into a cell, and, if replication is desired, which is replicable and viable in the relevant host can be used.

The nucleic acid sequence in the expression vector is operatively linked to an appropriate transcription control sequence (promoter) to direct mRNA synthesis. Examples

of such promoters include: LTR or SV40 promoter, *E. coli* lac or trp promoter, phage lambda P<sub>L</sub> promoter, and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation, and a transcription terminator. The vector optionally includes  
5 appropriate sequences for amplifying expression. In addition, the expression vectors optionally comprise one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells, such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in, e.g., *E. coli*.

The vector containing the appropriate DNA sequence as described above, as  
10 well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein. Examples of appropriate expression hosts include: bacterial cells, such as *E. coli*, *Streptomyces*, and *Salmonella typhimurium*; fungal cells, such as *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Neurospora crassa*; insect cells such as *Drosophila* and *Spodoptera frugiperda*; mammalian  
15 cells such as CHO, COS, BHK, HEK 293 or Bowes melanoma; plant cells, etc. It is understood that not all cells or cell lines need to be capable of producing fully functional lipase homologues; for example, antigenic fragments of lipase can be produced in a bacterial or other expression system. The invention is not limited by the host cells employed.

In bacterial systems, a number of expression vectors may be selected  
20 depending upon the use intended for the lipase homologue. For example, when large quantities of lipase homologue, or fragments thereof, are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the novel  
25 lipase coding sequence can be ligated into the vector in-frame with sequences for the amino-terminal Met and the subsequent 7 residues of beta-galactosidase so that a hybrid (or fusion) protein is produced; pIN vectors (Van Heeke & Schuster (1989) *J Biol Chem* 264:5503-5509); pET vectors (Novagen, Madison WI); and the like.

Similarly, in the yeast *Saccharomyces cerevisiae* a number of vectors  
30 containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH

may be used for production of the lipase homologue proteins of the invention. For reviews, see Ausubel et al. (*supra*) and Grant et al. (1987; Methods in Enzymology 153:516-544).

In mammalian host cells, a number expression systems, such as viral-based systems, can be utilized. In cases where an adenovirus is used as an expression vector, a coding sequence is optionally ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing lipase homologues in infected host cells (Logan and Shenk (1984) Proc Natl Acad Sci 81:3655-3659). In addition, transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

#### Additional Expression Elements

Specific initiation signals can aid in efficient translation of a lipase homologue coding sequence. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where lipase homologue coding sequence, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be positioned in the correct reading frame to ensure translation of the entire insert to generate the desired polypeptide. Exogenous transcriptional and/or translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use (Scharf D et al. (1994) Results Probl Cell Differ 20:125-62; Bittner et al. (1987) Methods in Enzymol 153:516-544).

#### Secretion/Localization Sequences

Polynucleotides of the invention can also be fused, for example, in-frame to nucleic acid encoding a secretion/localization sequence, to target polypeptide expression to a desired cellular compartment, membrane, or organelle, or to direct polypeptide secretion to the periplasmic space or into the cell culture media. Such sequences are known to those of skill, and include secretion leader peptides, organelle targeting sequences (e.g., nuclear

localization sequences, ER retention signals, mitochondrial transit sequences, chloroplast transit sequences), membrane localization/anchor sequences (e.g., stop transfer sequences, GPI anchor sequences), and the like.

#### Expression Hosts

5           In a further embodiment, the present invention relates to host cells containing the above-described constructs, e.g., vectors comprising lipase homologues. The host cell can be a eukaryotic cell, such as a mammalian cell, a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell (e.g., an *E. coli* cell). Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-  
10 Dextran mediated transfection, electroporation, or other common techniques (Davis, L., Dibner, M., and Battey, I. (1986) *Basic Methods in Molecular Biology*, Sambrook and Ausubel, *supra*).

A host cell strain is optionally chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such  
15 modifications of the protein include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a precursor form into a mature form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as COS, CHO, HeLa, BHK, MDCK, 293, WI38, etc. have specific cellular machinery and characteristic mechanisms for  
20 such post-translational activities and can be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression can be used. For example, cell lines which stably express a polypeptide of the invention are transduced using expression vectors which contain viral origins of replication  
25 or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. For example, resistant clumps of stably transformed cells can be  
30 proliferated using tissue culture techniques appropriate to the cell type.

Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell can be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding lipase homologues of the invention can be designed with signal sequences which direct secretion of the polypeptides through a prokaryotic or eukaryotic cell membrane.

#### Additional Polypeptide Sequences

The polynucleotides of the present invention may also comprise a coding sequence fused in-frame to a marker sequence which, e.g., facilitates purification of the encoded polypeptide. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, a sequence which binds glutathione (e.g., GST), a hemagglutinin (HA) tag (corresponding to an epitope derived from the influenza hemagglutinin protein; Wilson, I., et al. (1984) Cell 37:767), maltose binding protein sequences, the FLAG epitope utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle, WA), and the like. The inclusion of a protease-cleavable polypeptide linker sequence between the purification domain and the lipase homologue sequence is useful to facilitate purification.

For example, one expression vector contemplated for use in the compositions and methods described herein provides for expression of a fusion protein comprising a polypeptide of the invention fused to a polyhistidine region separated by an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography, as described in Porath et al. (1992) Protein Expression and Purification 3:263-281) while the enterokinase cleavage site provides a means for separating the lipase homologue polypeptide from the fusion protein. pGEX vectors (Promega; Madison, WI) can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from the culture medium or from lysed cells by adsorption to ligand-agarose



beads (e.g., glutathione-agarose in the case of GST-fusions) followed by elution in the presence of free ligand.

#### Polypeptide Production and Recovery

Following transduction of a suitable host cell line or strain and growth of the  
5 host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. The secreted polypeptide product is then recovered from the culture medium. Alternatively, cells can be harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Eukaryotic or  
10 microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, or other methods, which are well know to those skilled in the art.

As noted, many references are available for the culture and production of many cells, including cells of bacterial, plant, animal (especially mammalian) and  
15 archebacterial origin. *See, e.g., Sambrook, Ausubel, and Berger (all supra), as well as Freshney (1994) Culture of Animal Cells, a Manual of Basic Technique, third edition, Wiley-Liss, New York and the references cited therein; Doyle and Griffiths (1997) Mammalian Cell Culture: Essential Techniques John Wiley and Sons, NY; Humason (1979) Animal Tissue Techniques, fourth edition W.H. Freeman and Company; and Ricciardelli, et al., (1989) In vitro Cell Dev Biol 25:1016-1024. For plant cell culture and regeneration, see, e.g., Payne et al. (1992) Plant Cell and Tissue Culture in Liquid Systems John Wiley & Sons, Inc. New York, NY; Gamborg and Phillips (eds.) (1995) Plant Cell, Tissue and Organ Culture; Fundamental Methods Springer Lab Manual, Springer-Verlag (Berlin Heidelberg New York) and Plant Molecular Biology (1993) R. R. D. Croy, Ed. Bios Scientific Publishers, Oxford,  
20 U.K. ISBN 0 12 198370 6. Cell culture media in general are set forth in Atlas and Parks (eds.) The Handbook of Microbiological Media (1993) CRC Press, Boca Raton, FL. Additional information for cell culture is found in available commercial literature such as the Life Science Research Cell Culture Catalogue (1998) from Sigma- Aldrich, Inc (St Louis, MO) ("Sigma-LSRCCC") and, e.g., the Plant Culture Catalogue and supplement (1997) also  
30 from Sigma-Aldrich, Inc (St Louis, MO) ("Sigma-PCCS").*

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by any of a number of methods well known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography (e.g., using any of the tagging systems noted herein), hydroxylapatite chromatography, and lectin chromatography. Protein refolding steps can be used, as desired, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed in the final purification steps. In addition to the references noted above, a variety of purification methods are well known in the art, including, e.g., those set forth in Sandana (1997) *Bioseparation of Proteins*, Academic Press, Inc.; and Bollag et al. (1996) *Protein Methods, 2<sup>nd</sup> Edition* Wiley-Liss, NY; Walker (1996) *The Protein Protocols Handbook* Humana Press, NJ, Harris and Angal (1990) *Protein Purification Applications: A Practical Approach* IRL Press at Oxford, Oxford, England; Harris and Angal *Protein Purification Methods: A Practical Approach* IRL Press at Oxford, Oxford, England; Scopes (1993) *Protein Purification: Principles and Practice 3<sup>rd</sup> Edition* Springer Verlag, NY; Janson and Ryden (1998) *Protein Purification: Principles, High Resolution Methods and Applications*, Second Edition Wiley-VCH, NY; and Walker (1998) *Protein Protocols on CD-ROM* Humana Press, NJ.

#### In vitro Expression Systems

Cell-free transcription/translation systems can also be employed to produce polypeptides comprising lipase homologues, and fragments thereof, using DNAs or RNAs of the present invention. Several such systems are commercially available. A general guide to in vitro transcription and translation protocols is found in Tymms (1995) In vitro Transcription and Translation Protocols: Methods in Molecular Biology Volume 37, Garland Publishing, NY.

#### Modified Amino Acids

Polypeptides of the invention can contain one or more modified amino acid. The presence of modified amino acids can be advantageous in, for example, (a) increasing polypeptide serum half-life, (b) reducing polypeptide antigenicity, and (c) increasing polypeptide storage stability. Amino acid(s) are modified, for example, co-translationally or

post-translationally during recombinant production (e.g., N-linked glycosylation at N-X-S/T motifs during expression in mammalian cells) or modified by synthetic means.

Non-limiting examples of a modified amino acid include a glycosylated amino acid, a sulfated amino acid, a prenylated (e.g., farnesylated, geranylgeranylated) amino acid, an acetylated amino acid, an acylated amino acid, a PEG-ylated amino acid, a biotinylated amino acid, a carboxylated amino acid, a phosphorylated amino acid, and the like, as well as amino acids modified by conjugation to, e.g., lipid moieties or other organic derivatizing agents. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature. Example protocols are found in Walker (1998) *Protein Protocols on CD-ROM* Human Press, Towata, NJ.

#### IN VIVO USES

Polynucleotides which encode a lipase homologue of the invention, or complements of the polynucleotides (i.e., antisense polynucleotides), are optionally administered to a cell to accomplish a therapeutically useful process or to express a therapeutically useful product. These in vivo applications, including gene therapy, include a multitude of techniques by which gene expression can be altered in cells. Such methods include, for instance, the introduction of genes for expression of, e.g., therapeutically and/or prophylactically useful polypeptides, such as the lipase homologues of the present invention to, e.g., hydrolyze ester bonds of lipids, e.g., in the treatment of, e.g., Crohn's disease, etc.

#### In Vivo Polypeptide Expression

Polynucleotides encoding lipase homologue polypeptides of the invention are useful for in vivo therapeutic applications, including prophylactic applications, using techniques well known to those skilled in the art. For example, cultured cells are engineered ex vivo with a polynucleotide (DNA or RNA), with the engineered cells then being returned to the patient. Cells may also be engineered in vivo for expression of a polypeptide in vivo. As noted, and as described in more detail below, lipase production is also useful for a variety of industrial processes, including lipid degradation, and regio- or stereo-selective reaction with lipids.

A number of viral vectors suitable for organismal in vivo transduction and expression are known. Such vectors include retroviral vectors (*see*, Miller (1992) Curr Top

Microbiol Immunol 158:1-24; Salmons and Gunzburg (1993) Human Gene Therapy 4:129-141; Miller et al. (1994) Methods in Enzymology 217: 581-599) and adeno-associated vectors (reviewed in Carter (1992) Curr Opin Biotech 3: 533-539; Muzyczka (1992) Curr Top Microbiol Immunol 158: 97-129). Other viral vectors that are used include adenoviral  
5 vectors, herpes viral vectors and Sindbis viral vectors, as generally described in, e.g., Jolly (1994) Cancer Gene Therapy 1:51-64; Latchman (1994) Molec Biotechnol 2:179-195; and Johanning et al. (1995) Nucl Acids Res 23:1495-1501.

Gene therapy provides methods for combating chronic infectious diseases (e.g., HIV infection, viral hepatitis), as well as non-infectious diseases including cancer and  
10 some forms of congenital defects such as enzyme deficiencies. Several approaches for introducing nucleic acids into cells in vivo, ex vivo and in vitro have been used. These include liposome based gene delivery (Debs and Zhu (1993) WO 93/24640 and U.S. Pat. No. 5,641,662; Mannino and Gould-Fogerite (1988) BioTechniques 6(7): 682-691; Rose, U.S. Pat No. 5,279,833; Brigham (1991) WO 91/06309; and Felgner et al. (1987) Proc Natl Acad  
15 Sci USA 84: 7413-7414); Brigham et al. (1989) Am J Med Sci, 298:278-281; Nabel et al. (1990) Science, 249:1285-1288; Hazinski et al. (1991) Am J Resp Cell Molec Biol, 4:206-209; and Wang and Huang (1987) Proc Natl Acad Sci USA, 84:7851-7855); adenoviral vector mediated gene delivery, e.g., to treat cancer (see, e.g., Chen et al. (1994) Proc Natl  
Acad Sci USA 91: 3054-3057; Tong et al. (1996) Gynecol Oncol 61: 175-179; Clayman et  
20 al. (1995) Cancer Res 5: 1-6; O'Malley et al. (1995) Cancer Res 55: 1080-1085; Hwang et al. (1995) Am J Respir Cell Mol Biol 13: 7-16; Haddada et al. (1995) Curr Top Microbiol Immunol 199 (Pt. 3): 297-306; Addison et al. (1995) Proc Natl Acad Sci USA 92: 8522-8526; Colak et al. (1995) Brain Res 691: 76-82; Crystal (1995) Science 270: 404-410; Elshami et al. (1996) Human Gene Ther 7: 141-148; Vincent et al. (1996) J Neurosurg 85:  
25 648-654), and many other diseases. Replication-defective retroviral vectors harboring therapeutic polynucleotide sequence as part of the retroviral genome have also been used, particularly with regard to simple MuLV vectors. See, e.g., Miller et al. (1990) Mol Cell Biol 10:4239 (1990); Kolberg (1992) J NIH Res 4:43, and Cornetta et al. (1991) Hum Gene Ther 2:215). Nucleic acid transport coupled to ligand-specific, cation-based transport systems (Wu and Wu (1988) J Biol Chem, 263:14621-14624) have also been used. Naked DNA  
30

expression vectors have also been described (Nabel et al. (1990), *supra*); Wolff et al. (1990) *Science*, 247:1465-1468). In general, these approaches can be adapted to the invention by incorporating nucleic acids encoding the lipase homologues herein into the appropriate vectors.

5                   General texts which describe gene therapy protocols, which can be adapted to the present invention by introducing the nucleic acids of the invention into patients, include Robbins (1996) *Gene Therapy Protocols*, Humana Press, NJ, and Joyner (1993) *Gene Targeting: A Practical Approach*, IRL Press, Oxford, England.

#### Antisense Technology

10                   In addition to expression of the nucleic acids of the invention as gene replacement nucleic acids, the nucleic acids are also useful for sense and antisense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, once expression of the nucleic acid is no longer desired in the cell. Similarly, the nucleic acids of the invention, or subsequences or antisense sequences thereof, can also be  
15                   used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997) *Antisense Technology: A Practical Approach* IRL Press at Oxford University, Oxford, England, and in Agrawal (1996) *Antisense Therapeutics* Humana Press, NJ, and the references cited therein.

#### Pharmaceutical Compositions

20                   The polynucleotides of the invention may be employed for therapeutic uses in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the compound, and a pharmaceutically acceptable carrier or excipient. Such a carrier or excipient includes, but is not limited to, saline, buffered saline,  
25                   dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration. Methods of administering nucleic acids and proteins are well known in the art, and further discussed below.

#### Use as Probes

30                   Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at

least 20, 30, or 50 bases, which hybridize under highly stringent conditions to lipase a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like.

### SEQUENCE VARIATIONS

5

#### Silent Variations

It will be appreciated by those skilled in the art that due to the degeneracy of the genetic code, a multitude of nucleic acids sequences encoding novel lipase polypeptides of the invention may be produced, some which may bear minimal sequence homology to the nucleic acid sequences explicitly disclosed herein.

10

Table 1  
Codon Table

Amino acids			Codon						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Cysteine	Cys	C	UGC	UGU					
Aspartic acid	Asp	D	GAC	GAU					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	UUC	UUU					
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Methionine	Met	M	AUG						
Asparagine	Asn	N	AAC	AAU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Valine	Val	V	GUA	GUC	GUG	GUU			
Tryptophan	Trp	W	UGG						
Tyrosine	Tyr	Y	UAC	UAU					

For instance, inspection of the codon table (Table 1) shows that codons AGA, AGG, CGA, CGC, CGG, and CGU all encode the amino acid arginine. Thus, at every position in the nucleic acids of the invention where an arginine is specified by a codon, the

codon can be altered to any of the corresponding codons described above without altering the encoded polypeptide. It is understood that U in an RNA sequence corresponds to T in a DNA sequence.

Using, as an example, the nucleic acid sequence of clone 1f15(g2) corresponding to nucleotides 2-16 of SEQ ID NO: 21, GAA CAC AAT CCA GTT, a silent variation of this sequence includes GAG CAT AAC CCC GTG, both of which sequences encode the amino acid sequence EHNPV, which corresponds to amino acids 1-5 of SEQ ID NO: 75.

Such "silent variations" are one species of "conservatively modified variations", discussed below. One of skill will recognize that each codon in a nucleic acid (except AUG and UGG, which are ordinarily the only codons for methionine and tryptophan respectively) can be modified by standard techniques to encode a functionally identical polypeptide. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in any described sequence. The invention provides each and every possible variation of nucleic acid sequence encoding a polypeptide of the invention that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code (e.g., as set forth in Table 1) as applied to the nucleic acid sequence encoding a lipase homologue polypeptide of the invention. All such variations of every nucleic acid herein are specifically provided and described by consideration of the sequence in combination with the genetic code. One of skill is fully able to generate or select such variations based upon knowledge of the genetic code as well as considerations such as codon preferences of a specific organism chosen for expression of a polypeptide encoded by the nucleic acid.

#### Conservative Variations

"Conservatively modified variations" or, simply, "conservative variations" of a particular nucleic acid sequence refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or, where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. One of skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 4%, 2%

or 1%) in an encoded sequence are “conservatively modified variations” where the alterations result in the deletion of an amino acid, addition of an amino acid, or substitution of an amino acid with a chemically similar amino acid.

Conservative substitution tables providing functionally similar amino acids are well known in the art. Table 2 sets forth six groups which contain amino acids that are “conservative substitutions” for one another.

Table 2  
Conservative Substitution Groups

1	Alanine (A)	Serine (S)	Threonine (T)
2	Aspartic acid (D)	Glutamic acid (E)	
3	Asparagine (N)	Glutamine (Q)	
4	Arginine (R)	Lysine (K)	
5	Isoleucine (I)	Leucine (L)	Methionine (M) Valine (V)
6	Phenylalanine (F)	Tyrosine (Y)	Tryptophan (W)

10

Thus, “conservatively substituted variations” of a listed polypeptide sequence of the present invention include substitutions of a small percentage, typically less than 5%, more typically less than 4%, 3%, 2% or 1%, of the amino acids of the polypeptide sequence, with a conservatively selected amino acid of the same conservative substitution group.

15

For example, a conservatively substituted variation of the polypeptide identified herein as SEQ ID NO: 75 will contain “conservative substitutions,” according to the six groups defined herein, in up to 9 residues (i.e., 5% of the amino acids) in the 180 amino acid polypeptide.

20

In a further example, if four conservative substitutions were localized in the region corresponding to amino acids 1-20 of SEQ ID NO: 75, examples of conservatively substituted variations of this region, EHNPV VMVHG IGGAS FNFAG, include:

DHNPV IMVHG MGGAS YNFAG and

DHQPV VVVHG IGGSS FNFSG

25

And the like, in accordance with the conservative substitutions listed in Table 2 (in the above example, conservative substitutions are underlined). Listing of a protein



sequence herein, in conjunction with the above substitution table, provides an express listing of all conservatively substituted proteins.

5 Finally, the addition of sequences which do not alter the encoded activity of a nucleic acid molecule, such as the addition of a non-functional sequence, is a conservative variation of the basic nucleic acid.

One of skill will appreciate that many conservative variations of the nucleic acid constructs which are disclosed yield a functionally identical construct. For example, as discussed above, owing to the degeneracy of the genetic code, "silent substitutions" (i.e., substitutions in a nucleic acid sequence which do not result in an alteration in an encoded polypeptide) are an implied feature of every nucleic acid sequence which encodes an amino acid. Similarly, "conservative amino acid substitutions," in one or a few amino acids in an amino acid sequence are substituted with different amino acids with highly similar properties, are also readily identified as being highly similar to a disclosed construct. Such conservative variations of each disclosed sequence are a feature of the present invention.

15

#### Nucleic Acid Hybridization

Nucleic acids "hybridize" when they associate, typically in solution. Nucleic acids hybridize due to a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes*, part I, chapter 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," (Elsevier, New York), as well as in Ausubel, *supra*, Hames and Higgins (1995) *Gene Probes 1*, IRL Press at Oxford University Press, Oxford, England (Hames and Higgins 1) and Hames and Higgins (1995) *Gene Probes 2*, IRL Press at Oxford University Press, Oxford, England (Hames and Higgins 2) provide details on the synthesis, labeling, detection and quantification of DNA and RNA, including oligonucleotides.

25

"Stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments, such as Southern and northern hybridizations, are sequence dependent, and are different under different environmental parameters. An extensive guide

to the hybridization of nucleic acids is found in Tijssen (1993), *supra*, and in Hames and Higgins 1 and Hames and Higgins 2, *supra*.

For purposes of the present invention, generally, "highly stringent" hybridization and wash conditions are selected to be about 5° C or less lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH (as noted below, highly stringent conditions can also be referred to in comparative terms). The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the test sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the  $T_m$  for a particular probe.

The  $T_m$  temperature of the nucleic acid duplexes indicates the temperature at which the duplex is 50% denatured under the given conditions and represents a direct measure of the stability of the nucleic acid hybrid. Thus, the  $T_m$  corresponds to the temperature corresponding to the midpoint in transition from helix to random coil; it depends on length, nucleotide composition, and ionic strength for long stretches of nucleotides.

After hybridization, unhybridized nucleic acid material can be removed by a series of washes, the stringency of which can be adjusted depending upon the desired results. Low stringency washing conditions (e.g., using higher salt and lower temperature) increases sensitivity, but can produce nonspecific hybridization signals and high background signals. Higher stringency conditions (e.g., using lower salt and higher temperature that is closer to the hybridization temperature) lowers the background signal, typically with only the specific signal remaining. See, Rapley, R. and Walker, J.M. eds., *Molecular Biotechnology Handbook* (Humana Press, Inc. 1998) (hereinafter "Rapley and Walker"), which is incorporated herein by reference in its entirety for all purposes.

The  $T_m$  of a DNA-DNA duplex can be estimated using the following equation:

$$T_m (\text{°C}) = 81.5^\circ\text{C} + 16.6 (\log_{10}M) + 0.41 (\%G + C) - 0.72 (\%f) - 500/n,$$

where  $M$  is the molarity of the monovalent cations (usually  $\text{Na}^+$ ),  $(\%G + C)$  is the percentage of guanosine (G) and cytosine (C) nucleotides,  $(\%f)$  is the percentage of formamide and  $n$  is the number of nucleotide bases (i.e., length) of the hybrid. See, Rapley and Walker, *supra*.

The  $T_m$  of an RNA-DNA duplex can be estimated as follows:

$T_m$  (°C) = 79.8°C + 18.5 (log<sub>10</sub>M) + 0.58 (%G + C) – 11.8(%G + C)<sup>2</sup> – 0.56 (%f) – 820/n, where M is the molarity of the monovalent cations (usually Na<sup>+</sup>), (%G + C) is the percentage of guanosine (G) and cytosine (C) nucleotides, (%f) is the percentage of formamide and n is the number of nucleotide bases (i.e., length) of the hybrid. *Id.* Equations 5 1 and 2 are typically accurate only for hybrid duplexes longer than about 100-200 nucleotides. *Id.*

The  $T_m$  of nucleic acid sequences shorter than 50 nucleotides can be calculated as follows:

$$T_m$$
 (°C) = 4(G + C) + 2(A + T),

10 where A (adenine), C, T (thymine), and G are the numbers of the corresponding nucleotides.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of stringent wash conditions is a 0.2x 15 SSC wash at 65°C for 15 minutes (*see* Sambrook, *supra* for a description of SSC buffer). Often the high stringency wash is preceded by a low stringency wash to remove background probe signal. An example low stringency wash is 2x SSC at 40°C for 15 minutes.

In general, a signal to noise ratio of 2.5x-5x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific 20 hybridization. Detection of at least stringent hybridization between two sequences in the context of the present invention indicates relatively strong structural similarity or homology to, e.g., the nucleic acids of the present invention provided in the sequence listings herein.

As noted, “highly stringent” conditions are selected to be about 5° C or less lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength 25 and pH. Target sequences that are closely related or identical to the nucleotide sequence of interest (e.g., “probe”) can be identified under highly stringency conditions. Lower stringency conditions are appropriate for sequences that are less complementary. *See, e.g.,* Rapley and Walker, *supra*.

Comparative hybridization can be used to identify nucleic acids of the 30 invention, and this comparative hybridization method is a preferred method of distinguishing

nucleic acids of the invention. Detection of highly stringent hybridization between two nucleotide sequences in the context of the present invention indicates relatively strong structural similarity/homology to, e.g., the nucleic acids provided in the sequence listing herein. Highly stringent hybridization between two nucleotide sequences demonstrates a degree of similarity or homology of structure, nucleotide base composition, arrangement or order that is greater than that detected by stringent hybridization conditions. In particular, detection of highly stringent hybridization in the context of the present invention indicates strong structural similarity or structural homology (e.g., nucleotide structure, base composition, arrangement or order) to, e.g., the nucleic acids provided in the sequence listings herein. For example, it is desirable to identify test nucleic acids which hybridize to the exemplar nucleic acids herein under stringent conditions.

Thus, one measure of stringent hybridization is the ability to hybridize to one of the listed nucleic acids (e.g., nucleic acid sequences SEQ ID NO:1 to SEQ ID NO:54, and complementary polynucleotide sequences thereof) under highly stringent conditions (or very stringent conditions, or ultra-high stringency hybridization conditions, or ultra-ultra high stringency hybridization conditions). Stringent hybridization (including, e.g., highly stringent, ultra-high stringency, or ultra-ultra high stringency hybridization conditions) and wash conditions can easily be determined empirically for any test nucleic acid.

For example, in determining highly stringent hybridization and wash conditions, the hybridization and wash conditions are gradually increased (e.g., by increasing temperature, decreasing salt concentration, increasing detergent concentration and/or increasing the concentration of organic solvents, such as formamide, in the hybridization or wash), until a selected set of criteria are met. For example, the hybridization and wash conditions are gradually increased until a probe comprising one or more nucleic acid sequences selected from SEQ ID NO:1 to SEQ ID NO:54, and complementary polynucleotide sequences thereof, binds to a perfectly matched complementary target (again, a nucleic acid comprising one or more nucleic acid sequences selected from SEQ ID NO:1 to SEQ ID NO:54, and complementary polynucleotide sequences thereof), with a signal to noise ratio that is at least 2.5x, and optionally 5x or more as high as that observed for hybridization of the probe to an unmatched target. In this case, the unmatched target is a nucleic acid

corresponding to, e.g., a known lipase homologue, e.g., a lipase homologue nucleic acid (other than those in the accompanying sequence listing) that is present in a public database such as GenBank™ at the time of filing of the subject application. Examples of such unmatched target nucleic acids include, e.g., those represented by or which encode the following GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. It will be appreciated that the above GenBank accession numbers represent both amino acid and nucleic acid sequences. In the present application, such sequences should be read in context, e.g., when the context indicates an amino acid is to be considered, then the accession numbers that represent a nucleic acid should be interpreted as their amino acid translations and when the context indicates that a nucleic acid is intended, then the accession numbers representing amino acids should be interpreted as representing their corresponding nucleic acid. Additional such sequences can be identified in GenBank by one of skill.

A test nucleic acid is said to specifically hybridize to a probe nucleic acid when it hybridizes at least ½ as well to the probe as to the perfectly matched complementary target, i.e., with a signal to noise ratio at least ½ as high as hybridization of the probe to the target under conditions in which the perfectly matched probe binds to the perfectly matched complementary target with a signal to noise ratio that is at least about 5x-10x as high as that observed for hybridization to any of the unmatched target nucleic acids, e.g., represented by or which encode the following GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

Ultra high-stringency hybridization and wash conditions are those in which the stringency of hybridization and wash conditions are increased until the signal to noise ratio for binding of the probe to the perfectly matched complementary target nucleic acid is at least 10x as high as that observed for hybridization to any of the unmatched target nucleic acids, e.g., represented by or which encode the following GenBank accession numbers:

5 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, 10 D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. A target nucleic acid which hybridizes to a probe under such conditions, with a signal to noise ratio of at least  $\frac{1}{2}$  that of the perfectly matched complementary target nucleic acid is said to bind to the probe under ultra-high stringency conditions.

15 Similarly, even higher levels of stringency can be determined by gradually increasing the hybridization and/or wash conditions of the relevant hybridization assay. For example, those in which the stringency of hybridization and wash conditions are increased until the signal to noise ratio for binding of the probe to the perfectly matched complementary target nucleic acid is at least 10x, 20x, 50x, 100x, or 500x or more as high as

20 that observed for hybridization to any of the unmatched target nucleic acids represented by or which encode the following GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, 25 CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. A target nucleic acid which hybridizes to a probe under such conditions, with a signal to noise ratio of at least  $\frac{1}{2}$  that of the perfectly matched complementary target nucleic acid is said to bind to the probe under ultra-ultra-high stringency conditions.

Target nucleic acids which hybridize to the nucleic acids represented by SEQ ID NO:1 to SEQ ID NO:54 under high, ultra-high and ultra-ultra high stringency conditions are a feature of the invention. Examples of such nucleic acids include those with one or a few silent or conservative nucleic acid substitutions as compared to a given nucleic acid sequence.

Nucleic acids, such as man-made nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code, or when antisera generated against one or more of SEQ ID NO:55 to SEQ ID NO:108, which has been subtracted using the polypeptides represented by or which encode the following lipase related sequences in GenBank: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. Further details on immunological identification of polypeptides of the invention are found below.

In one aspect, the invention provides a nucleic acid which comprises a unique subsequence in a nucleic acid selected from SEQ ID NO:1 to SEQ ID NO:54. The unique subsequence is unique as compared to a nucleic acid corresponding to any of the sequences represented or which encode, e.g., by GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108, or related sequences present in GenBank as of the filing of this application. Alignment can be performed using the BLAST algorithm set to default parameters. Any unique subsequence is useful, e.g., as a probe to identify the nucleic acids of the invention.

Similarly, the invention includes a polypeptide which comprises a unique subsequence in a polypeptide selected from: SEQ ID NO:55 to SEQ ID NO:108. Here, the unique subsequence is unique as compared to a polypeptide corresponding to any of the sequences represented by or which encode GenBank accession numbers: 1I6WA, 1I6WB, 5 A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and 10 Z99108. Such unique subsequences can be determined by aligning any of SEQ ID NO:55 to SEQ ID NO:108 against the complete set of polypeptides represented by or which encode GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, 15 CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108 (the control polypeptides) (note that where the sequence corresponds to a non-translated sequence such as a pseudo gene, the corresponding polypeptide is generated simply by in silico translation of the nucleic acid 20 sequence into an amino acid sequence, where the reading frame is selected to correspond to the reading frame of lipase nucleic acids.

The invention also provides for target nucleic acids which hybridizes under stringent conditions to a unique coding oligonucleotide which encodes a unique subsequence in a polypeptide selected from: SEQ ID NO:55 to SEQ ID NO:108, wherein the unique 25 subsequence is unique as compared to a polypeptide corresponding to any of the control polypeptides (i.e., the above listed GenBank accession numbers). Unique sequences are determined as noted above.

In one example, the stringent conditions are selected such that a perfectly complementary oligonucleotide to the coding oligonucleotide hybridizes to the coding 30 oligonucleotide with at least about a 5-10x higher signal to noise ratio than for hybridization



of the perfectly complementary oligonucleotide to a control nucleic acid corresponding to any of the control polypeptides. Conditions can be selected such that higher ratios of signal to noise are observed in the particular assay which is used, e.g., about 15x, 20x, 30x, 50x or more. In this example, the target nucleic acid hybridizes to the unique coding oligonucleotide with at least a 2x higher signal to noise ratio (i.e., stringent conditions) as compared to hybridization of the control nucleic acid to the coding oligonucleotide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 20x, 30x, 50x or more. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

10

Percent Sequence Identity – Sequence Similarity

As noted above, the peptides employed in the subject invention need not be identical, but can be substantially identical, to the corresponding sequence of a lipase molecule or related molecule. The peptides can be subject to various changes, such as insertions, deletions, and substitutions, either conservative or non-conservative, where such changes might provide for certain advantages in their use. The polypeptides of the invention can be modified in a number of ways so long as they comprise a sequence substantially identical (as defined below) to a sequence in a lipase molecule.

15

Alignment and comparison of relatively short amino acid sequences (less than about 30 residues) is typically straightforward. Comparison of longer sequences can require more sophisticated methods to achieve optimal alignment of two sequences. Optimal alignment of sequences for aligning a comparison window can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv Appl Math 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J Mol Biol 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc Natl Acad Sci USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection, with the best alignment (i.e., resulting in the highest percentage of sequence similarity over the comparison window) generated by the various methods being selected.

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The term sequence identity means that two polynucleotide sequences are identical (i.e., on a nucleotide-by-nucleotide basis) over a window of comparison. The term “percentage of sequence identity” is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical residues occur in both sequences to yield the number of matched positions, dividing  
5 the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

As applied to polypeptides, the term substantial identity means that two  
10 peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights (described in more detail below), share at least about 70 percent sequence identity, or at least about 75 percent sequence identity, frequently at least about 80 percent sequence identity, often at least about 85 percent sequence identity, preferably at least about 90 percent sequence identity, more preferably at least about 95, 96, 97, 98 percent  
15 sequence identity or more (e.g., 99 percent or more sequence identity) over a designated comparison window, e.g., of at least 45 contiguous amino acids up to the entire length of the polypeptide sequence. Alternatively, parameters are set such that one or more sequences of the invention, e.g., SEQ ID NO:55 to SEQ ID NO:108 are identified by alignment to a query sequence selected from among SEQ ID NO:55 to SEQ ID NO:108, while sequences  
20 corresponding to unrelated polypeptides, e.g., corresponding to GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850,  
25 D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108, are not identified.

Preferably, residue positions which are not identical differ by conservative amino acid substitutions. Conservative amino acid substitution refers to the interchangeability of residues having similar side chains. For example, a group of amino  
30 acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group

of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

A preferred example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the FASTA algorithm, which is described in Pearson, W.R. & Lipman, D. J., (1988) Proc Natl Acad Sci USA 85:2444. See also, W. R. Pearson, (1996) Methods Enzymology 266:227-258. Preferred parameters used in a FASTA alignment of DNA sequences to calculate percent identity are optimized, BL50 Matrix 15: -5, k-tuple = 2; joining penalty = 40, optimization = 28; gap penalty -12, gap length penalty =-2; and width = 16.

Other preferred examples of algorithm that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., (1977) Nuc Acids Res 25:3389-3402 and Altschul et al., (1990) J Mol Biol 215:403-410, respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/)). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching

residues; always  $< 0$ ). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction is halted when: the cumulative alignment score falls off by the quantity  $X$  from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters  $W$ ,  $T$ , and  $X$  determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength ( $W$ ) of 11, an expectation ( $E$ ) of 10,  $M=5$ ,  $N=4$  and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation ( $E$ ) of 10, and the BLOSUM62 scoring matrix (*see*, Henikoff & Henikoff, (1989) Proc Natl Acad Sci USA 89:10915) uses alignments ( $B$ ) of 50, expectation ( $E$ ) of 10,  $M=5$ ,  $N=4$ , and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see*, e.g., Karlin & Altschul, (1993) Proc Natl Acad Sci USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability ( $P(N)$ ), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

Another example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percent sequence identity. It also plots a tree or dendrogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, (1987) J Mol Evol 35:351-360. The method used is similar to the method described by Higgins & Sharp, (1989) CABIOS 5:151-153. The program can align up to 300 sequences, each of a maximum length of 5,000 nucleotides or amino acids. The multiple alignment procedure begins with the pairwise alignment of the two most similar sequences, producing a cluster of two aligned sequences. This cluster is then aligned to the next most related sequence or cluster of aligned sequences. Two clusters of sequences are aligned by a simple extension of the pairwise

alignment of two individual sequences. The final alignment is achieved by a series of progressive, pairwise alignments. The program is run by designating specific sequences and their amino acid or nucleotide coordinates for regions of sequence comparison and by designating the program parameters. Using PILEUP, a reference sequence is compared to  
5 other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, e.g., version 7.0 (Devereaux et al., (1984) Nuc Acids Res 12:387-395).

Another preferred example of an algorithm that is suitable for multiple DNA  
10 and amino acid sequence alignments is the CLUSTALW program (Thompson, J. D. et al., (1994) Nuc Acids Res 22:4673-4680). CLUSTALW performs multiple pairwise comparisons between groups of sequences and assembles them into a multiple alignment based on homology. Gap open and Gap extension penalties were 10 and 0.05 respectively. For amino acid alignments, the BLOSUM algorithm can be used as a protein weight matrix  
15 (Henikoff and Henikoff, (1992) Proc Natl Acad Sci USA 89:10915-10919).

It will be understood by one of ordinary skill in the art, that the above discussion of search and alignment algorithms also applies to identification and evaluation of polynucleotide sequences, with the substitution of query sequences comprising nucleotide sequences, and where appropriate, selection of nucleic acid databases.

## 20 SUBSTRATES AND FORMATS FOR SEQUENCE RECOMBINATION

A variety of diversity generating protocols are available and described in the art. The procedures can be used separately, and/or in combination to produce one or more variants of a nucleic acid or set of nucleic acids, as well variants of encoded proteins. Individually and collectively, these procedures provide robust, widely applicable ways of  
25 generating diversified nucleic acids and sets of nucleic acids (including, e.g., nucleic acid libraries) useful, e.g., for the engineering or rapid evolution of nucleic acids, proteins, pathways, cells and/or organisms with new and/or improved characteristics.

While distinctions and classifications are made in the course of the ensuing discussion for clarity, it will be appreciated that the techniques are often not mutually

exclusive. Indeed, the various methods can be used singly or in combination, in parallel or in series, to access diverse sequence variants.

The result of any of the diversity generating procedures described herein can be the generation of one or more nucleic acids, which can be selected or screened for nucleic acids with or which confer desirable properties, or that encode proteins with or which confer desirable properties. Following diversification by one or more of the methods herein, or otherwise available to one of skill, any nucleic acids that are produced can be selected for a desired activity or property, e.g. lipase activity and/or enantioselective lipase activity or lipase activity against particular substrates. This can include identifying any activity that can be detected, for example, in an automated or automatable format, by any of the assays in the art, e.g., by any lipase activity assay (*see, infra*, for examples of lipase activity assays). A variety of related (or even unrelated) properties can be evaluated, in serial or in parallel, at the discretion of the practitioner.

Descriptions of a variety of diversity generating procedures for generating modified nucleic acid sequences encoding lipase homologues are found in the following publications and the references cited therein: Soong, N. et al. (2000) "Molecular breeding of viruses" Nat Genet 25(4):436-439; Stemmer, et al. (1999) "Molecular breeding of viruses for targeting and other clinical properties" Tumor Targeting 4:1-4; Ness et al. (1999) "DNA Shuffling of subgenomic sequences of subtilisin" Nature Biotechnology 17:893-896; Chang et al. (1999) "Evolution of a cytokine using DNA family shuffling" Nature Biotechnology 17:793-797; Minshull and Stemmer (1999) "Protein evolution by molecular breeding" Current Opinion in Chemical Biology 3:284-290; Christians et al. (1999) "Directed evolution of thymidine kinase for AZT phosphorylation using DNA family shuffling" Nature Biotechnology 17:259-264; Crameri et al. (1998) "DNA shuffling of a family of genes from diverse species accelerates directed evolution" Nature 391:288-291; Crameri et al. (1997) "Molecular evolution of an arsenate detoxification pathway by DNA shuffling," Nature Biotechnology 15:436-438; Zhang et al. (1997) "Directed evolution of an effective fucosidase from a galactosidase by DNA shuffling and screening" Proc Natl Acad Sci USA 94:4504-4509; Patten et al. (1997) "Applications of DNA Shuffling to Pharmaceuticals and Vaccines" Current Opinion in Biotechnology 8:724-733; Crameri et al. (1996) "Construction

and evolution of antibody-phage libraries by DNA shuffling" Nature Medicine 2:100-103; Cramer et al. (1996) "Improved green fluorescent protein by molecular evolution using DNA shuffling" Nature Biotechnology 14:315-319; Gates et al. (1996) "Affinity selective isolation of ligands from peptide libraries through display on a lac repressor 'headpiece dimer'" Journal of Molecular Biology 255:373-386; Stemmer (1996) "Sexual PCR and Assembly PCR" In: The Encyclopedia of Molecular Biology. VCH Publishers, New York. pp.447-457; Cramer and Stemmer (1995) "Combinatorial multiple cassette mutagenesis creates all the permutations of mutant and wildtype cassettes" BioTechniques 18:194-195; Stemmer et al., (1995) "Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides" Gene, 164:49-53; Stemmer (1995) "The Evolution of Molecular Computation" Science 270: 1510; Stemmer (1995) "Searching Sequence Space" Bio/Technology 13:549-553; Stemmer (1994) "Rapid evolution of a protein in vitro by DNA shuffling" Nature 370:389-391; and Stemmer (1994) "DNA shuffling by random fragmentation and reassembly: In vitro recombination for molecular evolution." Proc Natl Acad Sci USA 91:10747-10751.

Mutational methods of generating diversity include, for example, site-directed mutagenesis (Ling et al. (1997) "Approaches to DNA mutagenesis: an overview" Anal Biochem 254(2): 157-178; Dale et al. (1996) "Oligonucleotide-directed random mutagenesis using the phosphorothioate method" Methods Mol Biol 57:369-374; Smith (1985) "In vitro mutagenesis" Ann Rev Genet 19:423-462; Botstein & Shortle (1985) "Strategies and applications of in vitro mutagenesis" Science 229:1193-1201; Carter (1986) "Site-directed mutagenesis" Biochem J 237:1-7; and Kunkel (1987) "The efficiency of oligonucleotide directed mutagenesis" in Nucleic Acids & Molecular Biology (Eckstein, F. and Lilley, D.M.J. eds., Springer Verlag, Berlin)); mutagenesis using uracil containing templates (Kunkel (1985) "Rapid and efficient site-specific mutagenesis without phenotypic selection" Proc Natl Acad Sci USA 82:488-492; Kunkel et al. (1987) "Rapid and efficient site-specific mutagenesis without phenotypic selection" Methods in Enzymol 154, 367-382; and Bass et al. (1988) "Mutant Trp repressors with new DNA-binding specificities" Science 242:240-245); oligonucleotide-directed mutagenesis (Methods in Enzymol 100: 468-500 (1983); Methods in Enzymol 154: 329-350 (1987); Zoller & Smith (1982) "Oligonucleotide-directed

mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment" Nucleic Acids Res 10:6487-6500; Zoller & Smith (1983) "Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors" Methods in Enzymol 100:468-500; and Zoller & Smith (1987)

5 "Oligonucleotide-directed mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template" Methods in Enzymol 154:329-350); phosphorothioate-modified DNA mutagenesis (Taylor et al. (1985) "The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA" Nucl. Acids Res. 13: 8749-8764; Taylor et al. (1985) "The rapid generation of oligonucleotide-directed mutations at

10 high frequency using phosphorothioate-modified DNA" Nucl. Acids Res. 13: 8765-8787 (1985); Nakamaye & Eckstein (1986) "Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis" Nucl Acids Res 14: 9679-9698; Sayers et al. (1988) "Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis" Nucl Acids Res 16:791-802; and Sayers et al.

15 (1988) "Strand specific cleavage of phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide" Nucl Acids Res 16: 803-814); mutagenesis using gapped duplex DNA (Kramer et al. (1984) "The gapped duplex DNA approach to oligonucleotide-directed mutation construction" Nucl Acids Res 12: 9441-9456; Kramer & Fritz (1987) Methods in Enzymol "Oligonucleotide-directed construction of

20 mutations via gapped duplex DNA" 154:350-367; Kramer et al. (1988) "Improved enzymatic in vitro reactions in the gapped duplex DNA approach to oligonucleotide-directed construction of mutations" Nucl Acids Res 16: 7207; and Fritz et al. (1988) "Oligonucleotide-directed construction of mutations: a gapped duplex DNA procedure without enzymatic reactions in vitro" Nucl Acids Res 16: 6987-6999).

25 Additional suitable methods include point mismatch repair (Kramer et al. (1984) "Point Mismatch Repair" Cell 38:879-887), mutagenesis using repair-deficient host strains (Carter et al. (1985) "Improved oligonucleotide site-directed mutagenesis using M13 vectors" Nucl Acids Res 13: 4431-4443; and Carter (1987) "Improved oligonucleotide-directed mutagenesis using M13 vectors" Methods in Enzymol 154: 382-403), deletion

30 mutagenesis (Eghtedarzadeh & Henikoff (1986) "Use of oligonucleotides to generate large



deletions" Nucl Acids Res 14: 5115), restriction-selection and restriction-purification (Wells et al. (1986) "Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin" Phil. Trans. R. Soc. Lond. A 317: 415-423), mutagenesis by total gene synthesis (Nambiar et al. (1984) "Total synthesis and cloning of a gene coding for the ribonuclease S protein" Science 223: 1299-1301; Sakamar and Khorana (1988) "Total synthesis and expression of a gene for the a-subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin)" Nucl Acids Res 14: 6361-6372; Wells et al. (1985) "Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites" Gene 34:315-323; and Grundström et al. (1985) "Oligonucleotide-directed mutagenesis by microscale 'shot-gun' gene synthesis" Nucl Acids Res 13: 3305-3316), double-strand break repair (Mandecki (1986) "Oligonucleotide-directed double-strand break repair in plasmids of *Escherichia coli*: a method for site-specific mutagenesis" Proc Natl Acad Sci USA, 83:7177-7181; and Arnold (1993) "Protein engineering for unusual environments" Current Opinion in Biotechnology 4:450-455). Additional details on many of the above methods can be found in Methods in Enzymology Volume 154, which also describes useful controls for troubleshooting problems with various mutagenesis methods.

Additional details regarding various diversity generating methods can be found in the following U.S. patents, PCT publications and applications, and EPO publications: U.S. Pat. No. 5,605,793 to Stemmer (February 25, 1997), "Methods for In Vitro Recombination;" U.S. Pat. No. 5,811,238 to Stemmer et al. (September 22, 1998) "Methods for Generating Polynucleotides having Desired Characteristics by Iterative Selection and Recombination;" U.S. Pat. No. 5,830,721 to Stemmer et al. (November 3, 1998), "DNA Mutagenesis by Random Fragmentation and Reassembly;" U.S. Pat. No. 5,834,252 to Stemmer, et al. (November 10, 1998) "End-Complementary Polymerase Reaction;" U.S. Pat. No. 5,837,458 to Minshull, et al. (November 17, 1998), "Methods and Compositions for Cellular and Metabolic Engineering;" WO 95/22625, Stemmer and Cramer, "Mutagenesis by Random Fragmentation and Reassembly;" WO 96/33207 by Stemmer and Lipschutz "End Complementary Polymerase Chain Reaction;" WO 97/20078 by Stemmer and Cramer "Methods for Generating Polynucleotides having Desired Characteristics by Iterative Selection and Recombination;" WO 97/35966 by Minshull and Stemmer, "Methods and

Compositions for Cellular and Metabolic Engineering;" WO 99/41402 by Punnonen et al.  
"Targeting of Genetic Vaccine Vectors;" WO 99/41383 by Punnonen et al. "Antigen Library  
Immunization;" WO 99/41369 by Punnonen et al. "Genetic Vaccine Vector Engineering;"  
WO 99/41368 by Punnonen et al. "Optimization of Immunomodulatory Properties of Genetic  
5 Vaccines;" EP 752008 by Stemmer and Cramer, "DNA Mutagenesis by Random  
Fragmentation and Reassembly;" EP 0932670 by Stemmer "Evolving Cellular DNA Uptake  
by Recursive Sequence Recombination;" WO 99/23107 by Stemmer et al., "Modification of  
Virus Tropism and Host Range by Viral Genome Shuffling;" WO 99/21979 by Apt et al.,  
"Human Papillomavirus Vectors;" WO 98/31837 by del Cardayre et al. "Evolution of Whole  
10 Cells and Organisms by Recursive Sequence Recombination;" WO 98/27230 by Patten and  
Stemmer, "Methods and Compositions for Polypeptide Engineering;" WO 98/27230 by  
Stemmer et al., "Methods for Optimization of Gene Therapy by Recursive Sequence  
Shuffling and Selection," WO 00/00632, "Methods for Generating Highly Diverse  
Libraries," WO 00/09679, "Methods for Obtaining in Vitro Recombined Polynucleotide  
15 Sequence Banks and Resulting Sequences," WO 98/42832 by Arnold et al., "Recombination  
of Polynucleotide Sequences Using Random or Defined Primers," WO 99/29902 by Arnold  
et al., "Method for Creating Polynucleotide and Polypeptide Sequences," WO 98/41653 by  
Vind, "An in Vitro Method for Construction of a DNA Library," WO 98/41622 by Borchert  
et al., "Method for Constructing a Library Using DNA Shuffling," and WO 98/42727 by Pati  
20 and Zarling, "Sequence Alterations using Homologous Recombination;" WO 00/18906 by  
Patten et al., "Shuffling of Codon-Altered Genes;" WO 00/04190 by del Cardayre et al.  
"Evolution of Whole Cells and Organisms by Recursive Recombination;" WO 00/42561 by  
Cramer et al., "Oligonucleotide Mediated Nucleic Acid Recombination;" WO 00/42559 by  
Selifonov and Stemmer "Methods of Populating Data Structures for Use in Evolutionary  
25 Simulations;" WO 00/42560 by Selifonov et al., "Methods for Making Character Strings,  
Polynucleotides & Polypeptides Having Desired Characteristics;" WO 01/23401 by Welch et  
al., "Use of Codon-Variied Oligonucleotide Synthesis for Synthetic Shuffling;" and  
PCT/US01/06775 "Single-Stranded Nucleic Acid Template-Mediated Recombination and  
Nucleic Acid Fragment Isolation" by Affholter.

In brief, several different general classes of sequence modification methods, such as mutation, recombination, etc. are applicable to the production of lipase homologue nucleic acids encoding polypeptides with desired properties, and are set forth, e.g., in the references above. The following exemplify some of the different types of preferred formats  
5 for diversity generation in the context of the present invention, including, e.g., certain recombination based diversity generation formats.

Nucleic acids can be recombined in vitro by any of a variety of techniques discussed in the references above, including e.g., DNase digestion of nucleic acids to be recombined followed by ligation and/or PCR reassembly of the nucleic acids. For example,  
10 sexual PCR mutagenesis can be used in which random (or pseudo random, or even non-random) fragmentation of the DNA molecule is followed by recombination, based on sequence similarity, between DNA molecules with different but related DNA sequences, in vitro, followed by fixation of the crossover by extension in a polymerase chain reaction. This process and many process variants is described in several of the references above, e.g.,  
15 in Stemmer (1994) Proc Natl Acad Sci USA 91:10747-10751. Thus, one or more in vitro recombination procedure can be employed to generate a diverse set of lipase nucleic acids suitable for evaluation in any of a variety of assays designed to identify lipase nucleic acids encoding lipase polypeptides with desired properties. See, e.g., the lipase activity assays described *infra*.

20 Similarly, nucleic acids can be recursively recombined in vivo, e.g., by allowing recombination to occur between nucleic acids in cells. Many such in vivo recombination formats are set forth in the references noted above. Such formats optionally provide direct recombination between nucleic acids of interest, or provide recombination between vectors, viruses, plasmids, etc., comprising the nucleic acids of interest, as well as  
25 other formats. Details regarding such procedures are found in the references noted above. Thus, lipase nucleic acids can also be diversified in vivo prior to, or in concert with, screening and/or selection procedures to identify lipase polypeptides with desired properties.

Whole genome recombination methods can also be used in which whole genomes of cells or other organisms are recombined, optionally including spiking of  
30 genomic recombination mixtures with desired library components (e.g., genes corresponding

to the pathways of the present invention). These methods have many applications, including those in which the identity of a target gene is not known. Details on such methods are found, e.g., in WO 98/31837 by del Cardayre et al. "Evolution of Whole Cells and Organisms by Recursive Sequence Recombination;" and in, e.g., WO 00/04190 by del Cardayre et al., also  
5 entitled "Evolution of Whole Cells and Organisms by Recursive Sequence Recombination."

Synthetic recombination methods can also be used, in which oligonucleotides corresponding to targets of interest are synthesized and reassembled in PCR or ligation reactions which include oligonucleotides which correspond to more than one parental nucleic acid, thereby generating new recombined nucleic acids. Oligonucleotides can be made by  
10 standard nucleotide addition methods, or can be made, e.g., by tri-nucleotide synthetic approaches. Details regarding such approaches are found in the references noted above, including, e.g., WO 00/42561 by Cramer et al., "Oligonucleotide Mediated Nucleic Acid Recombination;" WO 01/23401 by Welch et al., "Use of Codon-Varied Oligonucleotide Synthesis for Synthetic Shuffling;" WO 00/42560 by Selifonov et al., "Methods for Making  
15 Character Strings, Polynucleotides and Polypeptides Having Desired Characteristics;" and WO 00/42559 by Selifonov and Stemmer "Methods of Populating Data Structures for Use in Evolutionary Simulations."

In silico methods of recombination can be effected in which genetic algorithms are used in a computer to recombine sequence strings which correspond to  
20 homologous (or even non-homologous) nucleic acids. The resulting recombined sequence strings are optionally converted into nucleic acids by synthesis of nucleic acids which correspond to the recombined sequences, e.g., in concert with oligonucleotide synthesis/gene reassembly techniques. This approach can generate random, partially random, or designed variants. Many details regarding in silico recombination, including the use of genetic  
25 algorithms, genetic operators and the like in computer systems, combined with generation of corresponding nucleic acids (and/or proteins), as well as combinations of designed nucleic acids and/or proteins (e.g., based on cross-over site selection) as well as designed, pseudo-random, or random recombination methods are described in WO 00/42560 by Selifonov et al., "Methods for Making Character Strings, Polynucleotides and Polypeptides Having  
30 Desired Characteristics" and WO 00/42559 by Selifonov and Stemmer "Methods of

Populating Data Structures for Use in Evolutionary Simulations.” Extensive details regarding *in silico* recombination methods are found in these applications. This methodology is generally applicable to the present invention in providing for recombination of character strings corresponding to lipase homologues *in silico* and/ or the generation of corresponding nucleic acids or proteins.

5 Many methods of accessing natural diversity, e.g., by hybridization of diverse nucleic acids or nucleic acid fragments to single-stranded templates, followed by polymerization and/or ligation to regenerate full-length sequences, optionally followed by degradation of the templates and recovery of the resulting modified nucleic acids can be similarly used. In one method employing a single-stranded template, the fragment population derived from the genomic library(ies) is annealed with partial, or, often approximately full length ssDNA or RNA corresponding to the opposite strand. Assembly of complex chimeric genes from this population is then mediated by nuclease-base removal of non-hybridizing fragment ends, polymerization to fill gaps between such fragments and subsequent single stranded ligation. The parental polynucleotide strand can be removed by digestion (e.g., if RNA or uracil-containing), magnetic separation under denaturing conditions (if labeled in a manner conducive to such separation) and other available separation/purification methods. Alternatively, the parental strand is optionally co-purified with the chimeric strands and removed during subsequent screening and processing steps.

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15  
20 Additional details regarding this approach are found, e.g., in “Single-Stranded Nucleic Acid Template-Mediated Recombination and Nucleic Acid Fragment Isolation” by Affholter, PCT/US01/06775.

In another approach, single-stranded molecules are converted to double-stranded DNA (dsDNA) and the dsDNA molecules are bound to a solid support by ligand-mediated binding. After separation of unbound DNA, the selected DNA molecules are released from the support and introduced into a suitable host cell to generate a library enriched sequences which hybridize to the probe. A library produced in this manner provides a desirable substrate for further diversification using any of the procedures described herein.

25

Any of the preceding general recombination formats can be practiced in a reiterative fashion (e.g., one or more cycles of mutation/recombination or other diversity generation methods, optionally followed by one or more selection methods) to generate a more diverse set of recombinant nucleic acids.

5 Mutagenesis employing polynucleotide chain termination methods have also been proposed (*see e.g.*, U.S. Patent No. 5,965,408, "Method of DNA reassembly by interrupting synthesis" to Short, and the references above), and can be applied to the present invention. In this approach, double stranded DNAs corresponding to one or more genes sharing regions of sequence similarity are combined and denatured, in the presence or  
10 absence of primers specific for the gene. The single stranded polynucleotides are then annealed and incubated in the presence of a polymerase and a chain terminating reagent (e.g., ultraviolet, gamma or X-ray irradiation; ethidium bromide or other intercalators; DNA binding proteins, such as single strand binding proteins, transcription activating factors, or histones; polycyclic aromatic hydrocarbons; trivalent chromium or a trivalent chromium salt;  
15 or abbreviated polymerization mediated by rapid thermocycling; and the like), resulting in the production of partial duplex molecules. The partial duplex molecules, e.g., containing partially extended chains, are then denatured and reannealed in subsequent rounds of replication or partial replication resulting in polynucleotides which share varying degrees of sequence similarity and which are diversified with respect to the starting population of DNA  
20 molecules. Optionally, the products, or partial pools of the products, can be amplified at one or more stages in the process. Polynucleotides produced by a chain termination method, such as described above, are suitable substrates for any other described recombination format.

Diversity also can be generated in nucleic acids or populations of nucleic acids using a recombinational procedure termed "incremental truncation for the creation of  
25 hybrid enzymes" ("ITCHY") described in Ostermeier et al. (1999) "A combinatorial approach to hybrid enzymes independent of DNA homology" *Nature Biotech* 17:1205. This approach can be used to generate an initial library of variants which can optionally serve as a substrate for one or more in vitro or in vivo recombination methods. *See also*, Ostermeier et al. (1999) "Combinatorial Protein Engineering by Incremental Truncation," *Proc Natl Acad*

Sci USA, 96: 3562-67; Ostermeier et al. (1999), "Incremental Truncation as a Strategy in the Engineering of Novel Biocatalysts," Biological and Medicinal Chemistry, 7: 2139-44.

Mutational methods which result in the alteration of individual nucleotides or groups of contiguous or non-contiguous nucleotides can be favorably employed to introduce nucleotide diversity. For example, mutagenesis procedures resulting in changes of one or more nucleotide can be used to produce any number of lipase variants of the present invention. Many mutagenesis methods are found in the above-cited references; additional details regarding mutagenesis methods can be found in following, which can also be applied to the present invention.

For example, error-prone PCR can be used to generate nucleic acid variants. Using this technique, PCR is performed under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product. Examples of such techniques are found in the references above and, e.g., in Leung et al. (1989) Technique 1:11-15 and Caldwell et al. (1992) PCR Methods 15 Applic 2:28-33. Similarly, assembly PCR can be used, in a process which involves the assembly of a PCR product from a mixture of small DNA fragments. A large number of different PCR reactions can occur in parallel in the same reaction mixture, with the products of one reaction priming the products of another reaction.

Oligonucleotide directed mutagenesis can be used to introduce site-specific mutations in a nucleic acid sequence of interest. Examples of such techniques are found in the references above and, e.g., in Reidhaar-Olson et al. (1988) Science, 241:53-57.

Similarly, cassette mutagenesis can be used in a process that replaces a small region of a double stranded DNA molecule with a synthetic oligonucleotide cassette that differs from the native sequence. The oligonucleotide can contain, e.g., completely and/or partially randomized native sequence(s).

Recursive ensemble mutagenesis is a process in which an algorithm for protein mutagenesis is used to produce diverse populations of phenotypically related mutants, members of which differ in amino acid sequence. This method uses a feedback mechanism to monitor successive rounds of combinatorial cassette mutagenesis. Examples of this approach are found in Arkin & Youvan (1992) Proc Natl Acad Sci USA 89:7811-7815.

Exponential ensemble mutagenesis can be used for generating combinatorial libraries with a high percentage of unique and functional mutants. Small groups of residues in a sequence of interest are randomized in parallel to identify, at each altered position, amino acids which lead to functional proteins. Examples of such procedures are found in  
5 Delegrave & Youvan (1993) Biotechnology Research 11:1548-1552.

In vivo mutagenesis can be used to generate random mutations in any cloned DNA of interest by propagating the DNA, e.g., in a strain of *E. coli* that carries mutations in one or more of the DNA repair pathways. These "mutator" strains have a higher random mutation rate than that of a wild-type parent. Propagating the DNA in one of these strains  
10 will eventually generate random mutations within the DNA. Such procedures are described in the references noted above.

Other procedures for introducing diversity into a genome, e.g. a bacterial, fungal, animal or plant genome can be used in conjunction with the above described and/or referenced methods. For example, in addition to the methods above, techniques have been  
15 proposed which produce nucleic acid multimers suitable for transformation into a variety of species (*see*, e.g., Schellenberger U.S. Patent No. 5,756,316 and the references above). Transformation of a suitable host with such multimers, consisting of genes that are divergent with respect to one another, (e.g., derived from natural diversity or through application of site directed mutagenesis, error prone PCR, passage through mutagenic bacterial strains, and the  
20 like), provides a source of nucleic acid diversity for DNA diversification, e.g., by an in vivo recombination process as indicated above.

Alternatively, a multiplicity of monomeric polynucleotides sharing regions of partial sequence similarity can be transformed into a host species and recombined in vivo by the host cell. Subsequent rounds of cell division can be used to generate libraries, members  
25 of which, include a single, homogenous population, or pool of monomeric polynucleotides. Alternatively, the monomeric nucleic acid can be recovered by standard techniques, e.g., PCR and/or cloning, and recombined in any of the recombination formats, including recursive recombination formats, described above.

Methods for generating multispecies expression libraries have been described  
30 (in addition to the reference noted above, *see*, e.g., Peterson et al. (1998) U.S. Pat. No.



5,783,431 "Methods for Generating and Screening Novel Metabolic Pathways," and Thompson, et al. (1998) U.S. Pat. No. 5,824,485 (Methods for Generating and Screening Novel Metabolic Pathways) and their use to identify protein activities of interest has been proposed (In addition to the references noted above, *see*, Short (1999) U.S. Pat. No. 5,958,672 "Protein Activity Screening of Clones Having DNA from Uncultivated Microorganisms"). Multispecies expression libraries include, in general, libraries comprising cDNA or genomic sequences from a plurality of species or strains, operably linked to appropriate regulatory sequences, in an expression cassette. The cDNA and/or genomic sequences are optionally randomly ligated to further enhance diversity. The vector can be a shuttle vector suitable for transformation and expression in more than one species of host organism, e.g., bacterial species, eukaryotic cells. In some cases, the library is biased by preselecting sequences which encode a protein of interest, or which hybridize to a nucleic acid of interest. Any such libraries can be provided as substrates for any of the methods herein described.

15               The above described procedures have been largely directed to increasing nucleic acid and/ or encoded protein diversity. However, in many cases, not all of the diversity is useful, e.g., functional, and contributes merely to increasing the background of variants that must be screened or selected to identify the few favorable variants. In some applications, it is desirable to preselect or prescreen libraries (e.g., an amplified library, a genomic library, a cDNA library, a normalized library, etc.) or other substrate nucleic acids prior to diversification, e.g., by recombination-based mutagenesis procedures, or to otherwise bias the substrates towards nucleic acids that encode functional products. For example, in the case of antibody engineering, it is possible to bias the diversity generating process toward antibodies with functional antigen binding sites by taking advantage of *in vivo* recombination events prior to manipulation by any of the described methods. For example, recombined CDRs derived from B cell cDNA libraries can be amplified and assembled into framework regions (e.g., Jirholt et al. (1998) "Exploiting sequence space: shuffling *in vivo* formed complementarity determining regions into a master framework" *Gene* 215: 471) prior to diversifying according to any of the methods described herein.

Libraries can be biased towards nucleic acids which encode proteins with desirable enzyme activities. For example, after identifying a clone from a library which exhibits a specified activity, the clone can be mutagenized using any known method for introducing DNA alterations. A library comprising the mutagenized homologues is then  
5 screened for a desired activity, which can be the same as or different from the initially specified activity. An example of such a procedure is proposed in Short (1999) U.S. Patent No. 5,939,250 for "Production of Enzymes Having Desired Activities by Mutagenesis." Desired activities can be identified by any method known in the art. For example, WO 99/10539 proposes that gene libraries can be screened by combining extracts from the gene  
10 library with components obtained from metabolically rich cells and identifying combinations which exhibit the desired activity. It has also been proposed (e.g., WO 98/58085) that clones with desired activities can be identified by inserting bioactive substrates into samples of the library, and detecting bioactive fluorescence corresponding to the product of a desired activity using a fluorescent analyzer, e.g., a flow cytometry device, a CCD, a fluorometer, or  
15 a spectrophotometer.

Libraries can also be biased towards nucleic acids which have specified characteristics, e.g., hybridization to a selected nucleic acid probe. For example, application WO 99/10539 proposes that polynucleotides encoding a desired activity (e.g., an enzymatic activity, for example: a lipase, an esterase, a protease, a glycosidase, a glycosyl transferase, a  
20 phosphatase, a kinase, an oxygenase, a peroxidase, a hydrolase, a hydratase, a nitrilase, a transaminase, an amidase or an acylase) can be identified from among genomic DNA sequences in the following manner. Single stranded DNA molecules from a population of genomic DNA are hybridized to a ligand-conjugated probe. The genomic DNA can be derived from either a cultivated or uncultivated microorganism, or from an environmental  
25 sample. Alternatively, the genomic DNA can be derived from a multi-cellular organism, or a tissue derived therefrom. Second strand synthesis can be conducted directly from the hybridization probe used in the capture, with or without prior release from the capture medium or by a wide variety of other strategies known in the art. Alternatively, the isolated single-stranded genomic DNA population can be fragmented without further cloning and

used directly in, e.g., a recombination-based approach, that employs a single-stranded template, as described above.

“Non-Stochastic” methods of generating nucleic acids and polypeptides are alleged in Short “Non-Stochastic Generation of Genetic Vaccines and Enzymes” WO 00/46344. These methods, including proposed non-stochastic polynucleotide reassembly and site-saturation mutagenesis methods can be applied to the present invention as well. Random or semi-random mutagenesis using doped or degenerate oligonucleotides is also described in, e.g., Arkin and Youvan (1992) "Optimizing nucleotide mixtures to encode specific subsets of amino acids for semi-random mutagenesis" Biotechnology 10:297-300; Reidhaar-Olson et al. (1991) "Random mutagenesis of protein sequences using oligonucleotide cassettes" Methods Enzymol 208:564-86; Lim and Sauer (1991) "The role of internal packing interactions in determining the structure and stability of a protein" J Mol Biol 219:359-76; Breyer and Sauer (1989) "Mutational analysis of the fine specificity of binding of monoclonal antibody 51F to lambda repressor" J Biol Chem 264:13355-60); and "Walk-Through Mutagenesis" (Crea, R; US Patents 5,830,650 and 5,798,208, and EP Patent 0527809 B1.

It will readily be appreciated that any of the above described techniques suitable for enriching a library prior to diversification can also be used to screen the products, or libraries of products, produced by the diversity generating methods.

Kits for mutagenesis, library construction and other diversity generation methods are also commercially available. For example, kits are available from, e.g., Stratagene (e.g., QuickChange™ site-directed mutagenesis kit; and Chameleon™ double-stranded, site-directed mutagenesis kit), Bio/Can Scientific, Bio-Rad (e.g., using the Kunkel method described above), Boehringer Mannheim Corp., Clontech Laboratories, DNA Technologies, Epicentre Technologies (e.g., 5 prime 3 prime kit); Genpak Inc, Lemargo Inc, Life Technologies (Gibco BRL), New England Biolabs, Pharmacia Biotech, Promega Corp., Quantum Biotechnologies, Amersham International plc (e.g., using the Eckstein method above), and Anglian Biotechnology Ltd. (e.g., using the Carter/Winter method above).

The above references provide many mutational formats, including recombination, recursive recombination, recursive mutation and combinations or

recombination with other forms of mutagenesis, as well as many modifications of these formats. Regardless of the diversity generation format that is used, the nucleic acids of the invention can be recombined (with each other, or with related (or even unrelated) sequences) to produce a diverse set of recombinant nucleic acids, including, e.g., sets of homologous nucleic acids, as well as corresponding polypeptides.

The current invention provides methods of producing modified or recombinant nucleic acids comprising mutating or recombining (including recursive recombination with one or more additional nucleic acid) a nucleic acid of the invention (or a fragment thereof), as well as the modified or recombinant nucleic acids that are produced by such method. The method optionally includes wherein the one or more additional nucleic acid encodes a polypeptide comprising lipase activity and/or enantioselective lipase activity (or an amino acid subsequence or fragment thereof). The recombination (e.g., recursive recombination) is optionally done in vitro or in vivo and optionally produces at least one library of recombinant nucleic acids, which comprises at least one polypeptide comprising lipase activity and/or enantioselective lipase activity (or a homologue thereof). Both the nucleic acid library produced and a population of cells comprising the library are provided by the invention, as are the modified or recombinant nucleic acids produced by the mutation/recombination and the cells which comprise such nucleic acids. The invention also includes a method of producing a polypeptide by introducing a nucleic acid of the invention (or a fragment thereof), which is operably linked to a regulatory sequence capable of directing expression of such nucleic acid into a polypeptide in at least a subset of a population of cells or their progeny and then expressing the polypeptide in the subset of the population (or their progeny). The polypeptide produced from such method is also part of the current invention. Such method optionally includes isolating the polypeptide from the cells and optionally includes expressing the polypeptide by culturing the population (or subset) in a nutrient medium under conditions where the regulatory sequence directs expression of the polypeptide encoded by the nucleic acid (again, wherein the polypeptide is optionally isolated or recovered from the cells and/or from the nutrient media (such culturing is optionally done in a bulk fermentation vessel). The cells used in such methods are optionally bacterial, eukaryotic (e.g., fungal cells, yeast cells, plant cells, insect cells, or

mammalian cells (e.g., fertilized oocytes, embryonic stem cells, pluripotent stem cells, etc.)). If mammalian cells are utilized, a transgenic animal is optionally regenerated from the cells and the polypeptide is optionally recovered from the transgenic animal or from a by-product of the transgenic animal such as milk.

## 5 HIGH THROUGHPUT SCREENING

High throughput screening formats are typically those formats which enable the efficient evaluation of a large number of samples, such as are associated with a library of nucleic acid or polypeptide sequences. Typically, a high throughput screening assay enables the evaluation of greater than 100, more commonly greater than 500, often greater than 1000  
10 or more samples in an efficient manner. A number of types of assays can be adapted to a high throughput format. For example, the throughput associated with a nucleic hybridization assay can be increased by adapting the assay from, e.g., electrophoretic separation of the subject nucleic acids followed by transfer to a nylon or nitrocellulose membrane and subsequent hybridization, to a "dot blot" format based on direct application of the subject  
15 nucleic acids to a membrane in an array with subsequent hybridization to a probe. The throughput can be further increased by robotic assistance, e.g., of the nucleic acid preparation and/or membrane application steps of the procedure. Similarly, many cell based assays can be reduced in scale, and increased in processing efficiency.

In addition to the nucleic acid screening methods indicated above, high  
20 throughput assays are used in the context of the present invention to measure functional activity of the nucleic acid and polypeptides described herein. One common format for cell based screening assays in a high throughput format is the multiwell microtiter plate although other formats are also suitably adapted to the present invention (e.g., microfluidic devices such as the HP/Agilent Technologies HP2100 and the Caliper HTS system: Caliper  
25 Technologies, Mountain View, California).

Standard microtiter plates are available with 96, 384 or 1536 wells, although even higher numbers of wells are also available. Well construction and materials can be selected according to the precise application. For example, well dimensions vary in shape, cross sectional area, depth and volume the choice of which can be influenced by such  
30 parameters as minimizing reagent use, or maximizing product recovery. Common materials

include a myriad of plastics, including polystyrene, polypropylene and the like. For some cell culture applications, it is desirable to use microtiter plates that have been pre-treated with agents that improve cell adherence or survival, e.g., poly-lysine, gelatin, etc.

Typically the plate dimensions are selected for compatibility with robotic loading and handling devices. Suitable robotic plate handling devices include, e.g., Multimek from Beckman Coulter; Q-BOTIII from Genetix; and the BioRobot #9600/9604 from Qiagen.

#### OTHER POLYNUCLEOTIDE COMPOSITIONS

The invention also includes compositions comprising two or more polynucleotides of the invention (e.g., as substrates for recombination). The composition can comprise a library of recombinant nucleic acids, where the library contains at least 2, 3, 5, 10, 20, 50, 100, 1,000 or 5,000 or more nucleic acids. The nucleic acids are optionally cloned into expression vectors, providing expression libraries.

The invention also includes compositions produced by digesting one or more polynucleotide of the invention with a restriction endonuclease, an RNase, or a DNase (e.g., as is performed in certain of the recombination formats noted above); and compositions produced by fragmenting or shearing one or more polynucleotide of the invention by mechanical means (e.g., sonication, vortexing, and the like), or by chemical cleavage (e.g., by incorporating nucleotide analogues subject to, e.g., photo-activated or other cleavage) which can also be used to provide substrates for recombination in the methods above. Similarly, compositions comprising sets of oligonucleotides corresponding to more than one nucleic acid of the invention are useful as recombination substrates and are a feature of the invention. For convenience, these fragmented, sheared, or synthesized oligonucleotide mixtures are referred to as fragmented nucleic acid sets.

Also included in the invention are compositions produced by incubating one or more of the fragmented nucleic acid sets in the presence of ribonucleotide- or deoxyribonucleotide triphosphates and a nucleic acid polymerase. This resulting composition forms a recombination mixture for many of the recombination formats noted above. The nucleic acid polymerase may be an RNA polymerase, a DNA polymerase, or an

RNA-directed DNA polymerase (e.g., a “reverse transcriptase”); the polymerase can be, e.g., a thermostable DNA polymerase (such as, VENT, TAQ, or the like).

#### LIPASE HOMOLOGUE POLYPEPTIDES

The invention provides isolated or recombinant lipase homologue polypeptides, referred to herein as “novel lipase polypeptides,” “lipase homologue polypeptides,” “lipase homologues,” or simply “novel lipases.” For example, an isolated or recombinant lipase homologue polypeptide of the invention includes a polypeptide comprising a sequence selected from SEQ ID NO: 55 to SEQ ID NO: 108, and conservatively modified variations thereof (as well as a fragment of such, which fragment can comprise lipase activity and/or enantioselective lipase activity) Additionally, the invention provides a polypeptide encoded by a polynucleotide sequence selected from SEQ ID NO: 1 through SEQ ID NO: 54 or a complementary polynucleotide sequence thereof, etc. Alignments of both nucleic acid and amino acid exemplary lipase homologue polypeptide sequences (for both newly isolated homologues and for newly created homologues) according to the invention are provided in Figures 3 through 6. Figure 3 depicts an alignment of exemplary novel lipase polynucleotides of the invention (SEQ ID NOS:1-20). The predicted boundary between the signal peptide coding region and the mature coding region is indicated by the arrow. Thus, a mature coding region or mature polypeptide, either as a polypeptide or as its encoding nucleic acid, of the invention comprises such an area as is delineated in, e.g., Figure 3, i.e., it does not include signal peptide regions, introductory 5’ regions or tailing 3’ regions such as a TGA stop, etc. Figure 4 depicts an alignment of exemplary novel lipase polynucleotides of the invention (SEQ ID NOS:21-54). The nucleotide sequences depicted in the figure represent predicted mature coding regions, each with an introductory 5’ “T” just prior to the start of the mature coding region, and ending with a 3’ “TGA” stop codon. Figure 5 depicts an alignment of exemplary novel lipase polypeptides of the invention (SEQ ID NOS:55-74). The predicted boundary between the signal peptide and the mature region is indicated by the arrow. The position numbering along the top of the alignments indicate the position relative to the start of the mature region. Figure 6 depicts an alignment of exemplary novel lipase polypeptides of the invention (SEQ ID NOS:75-108). The sequences shown represent the predicted mature region. The

alignments shown in Figures 3-6 were prepared using the CLUSTALW multiple sequence alignment program, a part of the Vector NTI version 6 sequence analysis software package (Informax, Bethesda, MD), using default parameters.

Another feature of the invention is an isolated or recombinant polypeptide  
5 encoded by a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length, or to a subsequence thereof comprising at least 100 residues or more, of a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54 (or a complementary sequence thereof) or a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108 (or a complementary  
10 sequence thereof) or a fragment thereof (from either SEQ ID NO: 1-54 or SEQ ID NO: 55-108 which fragment can comprise lipase activity and/or enantioselective lipase activity) provided that the sequences do not correspond to or encode any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623,  
15 AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

Various aspects of the current invention comprise an isolated or recombinant  
20 polypeptide comprising a sequence having at least 97% amino acid sequence identity to any one of SEQ ID NO: 75 to SEQ ID NO: 108. Such polypeptide can optionally comprise or exhibit lipase activity (e.g., it can degrade geranyl butyrate or neryl butyrate or both). Additionally, such polypeptide can exhibit enantioselectivity for geranyl butyrate over neryl butyrate. Such polypeptide that exhibits enantioselectivity for geranyl butyrate can comprise  
25 a sequence selected from: SEQ ID NO:76, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:86, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:78, SEQ ID NO:87, SEQ ID NO:100, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:88, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:103, or SEQ ID NO:106. Alternatively, the polypeptide can exhibit enantioselectivity for neryl  
30 butyrate over geranyl butyrate. Such polypeptide that exhibits enantioselectivity for neryl



butyrate over geranyl butyrate can comprise a sequence selected from: SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:105, SEQ ID NO:84, SEQ ID NO:91, SEQ ID NO:92, or SEQ ID NO:93.

5                   Furthermore, the polypeptide can comprise a polypeptide encoded by a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of a polynucleotide sequence selected from SEQ ID NO: 1-54 (or a complementary sequence thereof), or by a polynucleotide sequence encoding a polypeptide sequence selected from SEQ ID NO: 55-108 (or a complementary sequence  
10 thereof), and wherein the polypeptide comprises one or more of: Lys at position 1; Thr at position 14; Ser at position 17; Arg at position 22; Glu at position 26; Pro at position 31; Gly at position 33; Glu at position 34; Pro at position 35; Pro or Thr at position 37; Ser or Lys at position 41; Gly at position 42; Arg or Glu at position 43; Ala at position 61; Tyr at position 75; Gly at position 96; Ser at position 97; Thr at position 104; Ser at position 107; Ala at  
15 position 125; Gly at position 129; Val at position 134; Cys at position 138; Lys at position 141; Lys at position 146; Thr at position 156; Met at position 160; Arg at position 166; or His at position 177. Alternatively, the polypeptide can comprise one or more of: Lys at position 1; Thr at position 14; Ser at position 17; Arg at position 22; Glu at position 26; Pro at position 31; Gly at position 33; Glu at position 34; Pro at position 35; Pro or Thr at position  
20 37; Ser or Lys at position 41; Gly at position 42; Arg or Glu at position 43; Ala at position 61; Tyr at position 75; Gly at position 96; Ser at position 97; Thr at position 104; Ser at position 107; Ala at position 125; Gly at position 129; Val at position 134; Cys at position 138; Lys at position 141; Lys at position 146; Thr at position 156; Met at position 160; Arg at position 166; or His at position 177 (or an equivalent position to that of SEQ ID NO: 75).

25                   Such polypeptide can comprise or exhibit lipase activity or the ability to degrade geranyl butyrate, neryl butyrate, or both neryl and geranyl butyrate. The polypeptide can also exhibit enantioselectivity for geranyl butyrate over neryl butyrate. A polypeptide exhibiting enantioselectivity for geranyl butyrate over neryl butyrate can comprise one or more of: Arg at position 22; Gly at position 33; Ser or Lys at position 41; Arg at position 43;

Ser at position 107; Lys at position 141; Lys at position 146; Met at position 160; or His at position 177, or can comprise one or more of: Arg at position 43; or Ser at position 107.

Such polypeptide can alternatively comprise or exhibit enantioselectivity for neryl butyrate over geranyl butyrate. Such polypeptide can comprise one or more of: Ser at  
5 position 17; Arg at position 22; Pro at position 31; Gly at position 33; Ser or Lys at position 41; Lys at position 141; Lys at position 146; Met at position 160; Arg at position 166; or His at position 177, or, can comprise one or more of: Ser at position 17; Pro at position 31; or Arg at position 166.

In another aspect, the invention can comprise an isolated or recombinant  
10 polypeptide comprising a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 55, 61, 64, 65, 67, 68, 70, or 72. Alternatively, such polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 55, which polypeptide also can comprise a sequence selected from SEQ ID NO: 55, 58-62, 75-78, 80-88, or 94-108 (or the mature region thereof).  
15 Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 61, which polypeptide also can comprise a sequence selected from SEQ ID NO: 55, 57-62, 75-78, 80-90, or 93-108. Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 64, which polypeptide also can  
20 comprise a sequence selected from SEQ ID NO: 64, 71, or 72 (or the mature region thereof). Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 65, which polypeptide can also comprise a sequence selected from SEQ ID NO: 65, 66, or 73 (or a mature region thereof). Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid  
25 sequence identity to the mature region of SEQ ID NO: 67, which polypeptide can also comprise the sequence SEQ ID NO: 67 (or the mature region thereof). Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 68, which polypeptide can also comprise a sequence selected from SEQ ID NO: 68 or 101 (or the mature region thereof). Alternatively, the polypeptide  
30 can comprise a sequence having at least 94% amino acid sequence identity to the mature

region of SEQ ID NO: 70, which polypeptide can also comprise a sequence selected from SEQ ID NO: 63, 68-70, 82-83, 85-86, 96, or 101-102 (or the mature region thereof).

Alternatively, the polypeptide can comprise a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 72, which polypeptide can also

5 comprise a sequence selected from SEQ ID NO: 64, 71, or 72 (or a mature region thereof).

In another aspect, the invention can comprise an isolated or recombinant polypeptide comprising a sequence having at least 85% amino acid sequence identity to the mature region of SEQ ID NO: 74, which polypeptide can also comprise a sequence selected from SEQ ID NO: 63, 71-72, 74, or 79 (or a mature region thereof).

10 In yet another aspect, the invention can comprise an isolated or recombinant polypeptide comprising a sequence having at least 99% amino acid sequence identity to the mature region of SEQ ID NO: 56.

The extent of the region of identity or similarity can extend from a comparison window of at least 45 amino acids to the entire length of the lipase homologue polypeptide. In an embodiment, such polypeptides are identified by performing a sequence alignment with any one or more of SEQ ID NO: 55 to SEQ ID NO: 108 using BLASTP with default parameters set to the desired percentage identity. Alternatively, the default parameters can be set to identify polypeptide sequences with greater identity to one or more of SEQ ID NO: 55 to SEQ ID NO: 108.

20 Alternatively, polypeptides of the invention can be encoded by polynucleotides that correspond to any one, or part of SEQ ID NO: 1 to SEQ ID NO: 54 (or complementary polynucleotides thereof) and or a fragment thereof, which fragment can comprise lipase activity. The polypeptides of the invention can, likewise, be encoded by polynucleotides that hybridize under stringent or highly stringent conditions over

25 substantially the entire length of such polynucleotides, with the proviso that such sequences do not correspond to or encode any of the GenBank accession 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621,

30 CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903,

E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. Similarly, polypeptides that are encoded by subsequences of any such polynucleotides, e.g., a subsequence comprising at least about 45 contiguous amino acid residues, sometimes comprising at least about 45 contiguous amino acid residues, and in some cases comprising 45 contiguous amino acid residues of the polypeptide are also a feature of the invention. In some instances, such polypeptides are substantially identical to one or more of SEQ ID NO: 55 to SEQ ID NO: 108 over at least 45 contiguous amino acid residues with the proviso that such sequences do not correspond to or encode any of the GenBank accession numbers listed above. In other cases, the polypeptides, regardless of length, display lipase activity and/or enantioselective lipase activity.

The invention provides isolated or recombinant polypeptides encoded by a nucleic acid comprising a polynucleotide sequence selected from any of the following: a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54 (or a complementary polynucleotide sequence thereof); a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108 (or a complementary polynucleotide sequence thereof); a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the whole length of any of the previous described polynucleotides, or which hybridizes to a subsequence of the same, comprising at least 100 residues, again, with the proviso that none of the sequences corresponds to or is encoded by any of the GenBank accession numbers listed above; a polynucleotide sequence which comprises all, or a fragment of, any of the above described polynucleotides and which encodes a polypeptide comprising lipase activity and/or lipase enantioselective activity; a polynucleotide sequence encoding a polypeptide which comprises an amino acid sequence that is substantially identical over at least 45 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108, with the proviso that none of the sequences corresponds to or is encoded by any of the GenBank accession numbers listed above; or a polynucleotide sequence encoding a polypeptide comprising lipase activity and that is produced by mutating or recombining one or more of the polynucleotide sequences described above, yet again, with the proviso that none of the sequences corresponds to or is encoded by any of the GenBank accession numbers listed above. The invention also provides an isolated or recombinant

polypeptide as described above which comprises an amino acid sequence of any of SEQ ID NO: 55 to SEQ ID NO: 108.

Isolated or recombinant polypeptides as described above wherein the encoded polypeptide comprises lipase activity (e.g., against tributyrin, against tributyrin in DMF, 5 against tributyrin after heat treatment (i.e., after the polypeptide has been heat treated); and/or enantioselective lipase activity (e.g., against neryl- butyrate or geranyl- butyrate) are also provided. Optionally, such polypeptides as described can comprise lipase activity against novel substrates (i.e., substrates upon which typical wild-type lipases do not act) such as, e.g., methyl esters, pentadecanolide, or oxacyclotridecan. Optionally the isolated or 10 recombinant nucleic acid can encode a polypeptide which comprises enantioselective activity as well as comprising a polynucleotide sequence encoding a polypeptide with enantioselective lipase activity. Additionally, such isolated or recombinant polypeptides optionally are substantially identical over at least 45, at least 50, at least 75, at least 100, at least 125 , at least 150, at least 175, or at least 200 contiguous amino acids of any of the 15 above described polypeptides. Alternatively, such isolated or recombinant polypeptides is substantially identical over at least 180, at least 212, at least 213, or at least 215 contiguous amino acid residues of the above described polypeptide.

In various embodiments, the above described polypeptides comprise one or more of: a leader sequence, a precursor polypeptide, a secretion signal or a localization 20 signal, an epitope tag, a fusion protein comprising one or more additional amino acid sequences, a polypeptide purification subsequence (e.g., an epitope tag, a FLAG tag, a polyhistidine sequence, a GST fusion), an N-terminus methionine residue, or a modified amino acid (e.g., a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid 25 moiety or to an organic derivatizing agent).

A composition comprising one or more polypeptide comprising a modified amino acid and pharmaceutically acceptable excipient and a composition comprising one or more above described polypeptide with a pharmaceutically acceptable excipient are also provided. Additionally, the invention provides a polypeptide which comprises a unique 30 subsequence in a polypeptide selected from SEQ ID NO: 55 through SEQ ID NO: 108

wherein such subsequence is unique as compared to a polypeptide sequence which corresponds to an amino acid sequence (or which is encoded by a nucleic acid sequence) corresponding to any of GenBank accession numbers 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. Also provided is a polypeptide which is specifically bound by a polyclonal antisera raised against at least one antigen comprising at least one amino acid sequence from SEQ ID NO: 55 to SEQ ID NO: 108 (or a fragment thereof) where the antisera is subtracted with a polypeptide corresponding to an amino acid sequence (or which is encoded by a nucleic acid sequence) corresponding to any of GenBank accession numbers 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

In other aspects the invention includes an antibody or antisera produced by administering an above described polypeptide of the invention to a mammal and wherein the antibody or antisera specifically binds at least one antigen which comprises a polypeptide sequence (or fragment thereof) from SEQ ID NO: 55 to SEQ ID NO: 108 and which antibody or antisera does not specifically bind to a polypeptide encoded by a nucleic acid corresponding to, or an amino acid sequence corresponding to one or more of GenBank accession numbers 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957,

S23934, U78785, X95309, Z99105, and Z99108. In yet other aspects, the invention includes an antibody or antisera that specifically binds a polypeptide which comprises an amino acid sequence (or fragment thereof) from SEQ ID NO: 55 to SEQ ID NO: 108 and which antibody or antisera does not specifically bind to a peptide encoded by a nucleic acid  
5 corresponding to, or an amino acid sequence corresponding to one or more of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662,  
10 CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

#### Making Polypeptides

Recombinant methods for producing and isolating lipase homologue polypeptides of the invention are described above. In addition to recombinant production,  
15 the polypeptides can be produced by direct peptide synthesis using solid-phase techniques (see, e.g., Stewart et al. (1969) *Solid-Phase Peptide Synthesis*, WH Freeman Co, San Francisco; Merrifield J (1963) *J Am Chem So.* 85:2149-2154). Peptide synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster  
20 City, Calif.) in accordance with the instructions provided by the manufacturer. For example, subsequences can be chemically synthesized separately and combined using chemical methods to provide full-length lipase homologues. Fragments of the lipase polypeptides of the invention, as discussed herein, are also a feature of the invention and can be synthesized by using the procedures described above.

25 Polypeptides of the invention can be produced by introducing into a population of cells a nucleic acid of the invention, wherein the nucleic acid is operatively linked to a regulatory sequence effective to produce the encoded polypeptide, culturing the cells in a culture medium to produce the polypeptide, and optionally isolating the polypeptide from the cells or from the culture medium.

In another aspect, polypeptides of the invention can be produced by introducing into a population of cells a recombinant expression vector comprising at least one nucleic acid of the invention, wherein the at least one nucleic acid is operatively linked to a regulatory sequence effective to produce the encoded polypeptide, culturing the cells in a culture medium under suitable conditions to produce the polypeptide encoded by the expression vector, and optionally isolating the polypeptide from the cells or from the culture medium.

#### Using Polypeptides

##### Antibodies

In another aspect of the invention, a lipase homologue polypeptide of the invention is used to produce antibodies which have, e.g., diagnostic and/or therapeutic uses, e.g., related to the activity, distribution, and expression of lipase homologues.

Antibodies to lipase homologues of the invention can be generated by methods well known in the art. Such antibodies can include, but are not limited to, polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by an Fab expression library. Antibodies, i.e., those which block receptor binding, are especially preferred for therapeutic use.

Lipase homologue polypeptides for antibody induction do not require biological activity; however, the polypeptide or oligopeptide must be antigenic. Peptides used to induce specific antibodies can have an amino acid sequence consisting of at least 10 amino acids, preferably at least 15 or 20 amino acids. Short stretches of a lipase polypeptide can be fused with another protein, such as keyhole limpet hemocyanin (KLH), and antibody produced against the chimeric molecule.

Methods of producing polyclonal and monoclonal antibodies are known to those of skill in the art, and many antibodies are available. See, e.g., Coligan (1991) *Current Protocols in Immunology* Wiley/Greene, NY; and Harlow and Lane (1989) *Antibodies: A Laboratory Manual* Cold Spring Harbor Press, NY; Stites et al. (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Goding (1986) *Monoclonal Antibodies: Principles and Practice* (2d ed.) Academic Press, New York, NY; and Kohler and Milstein (1975) Nature 256: 495-497. Other suitable



techniques for antibody preparation include selection of libraries of recombinant antibodies in phage or similar vectors. See, Huse et al. (1989) Science 246: 1275-1281; and Ward, et al. (1989) Nature 341: 544-546. Specific monoclonal and polyclonal antibodies and antisera will usually bind with a  $K_D$  of at least about 0.1  $\mu\text{M}$ , preferably at least about 0.01  $\mu\text{M}$  or better, and most typically and preferably, 0.001  $\mu\text{M}$  or better.

Detailed methods for preparation of chimeric (humanized) antibodies can be found in U.S. Patent 5,482,856. Additional details on humanization and other antibody production and engineering techniques can be found in Borrebaeck (ed.) (1995) Antibody Engineering, 2<sup>nd</sup> Edition Freeman and Company, NY (Borrebaeck); McCafferty et al. (1996) Antibody Engineering, A Practical Approach IRL at Oxford Press, Oxford, England (McCafferty), and Paul (1995) Antibody Engineering Protocols Humana Press, Towata, NJ (Paul).

In one useful embodiment, this invention provides for fully humanized antibodies against the lipase homologues of the invention. Humanized antibodies are especially desirable in applications where the antibodies are used as therapeutics in vivo in human patients. Human antibodies consist of characteristically human immunoglobulin sequences. The human antibodies of this invention can be produced using a wide variety of methods (see, e.g., Larrick et al., U.S. Pat. No. 5,001,065, and Borrebaeck McCafferty and Paul, *supra*, for a review). In one embodiment, the human antibodies of the present invention are produced initially in trioma cells. Genes encoding the antibodies are then cloned and expressed in other cells, such as nonhuman mammalian cells. The general approach for producing human antibodies by trioma technology is described by Ostberg et al. (1983), Hybridoma 2: 361-367, Ostberg, U.S. Pat. No. 4,634,664, and Engelman et al., U.S. Pat. No. 4,634,666. The antibody-producing cell lines obtained by this method are called triomas because they are descended from three cells; two human and one mouse. Triomas have been found to produce antibody more stably than ordinary hybridomas made from human cells.

## SEQUENCE VARIATIONS

### Conservatively Modified Variations

Lipase homologue polypeptides of the present invention include

conservatively modified variations of the sequences disclosed herein as SEQ ID NO: 55 to  
5 SEQ ID NO: 108. Such conservatively modified variations comprise substitutions, additions  
or deletions which alter, add or delete a single amino acid or a small percentage of amino  
acids (typically less than about 5%, more typically less than about 4%, 3%, 2%, or 1%, or  
less) in any of SEQ ID NO: 55 to SEQ ID NO: 108.

For example, a conservatively modified variation (e.g., deletion) of the 180  
10 amino acid polypeptide identified herein as SEQ ID NO: 75 will have a length of at least 171  
amino acids, preferably at least 173 amino acids, preferably at least 175 amino acids, more  
preferably at least 177 amino acids, and still more preferably at least 179 amino acids,  
corresponding to a deletion of less than about 5%, 4%, 3%, 2%, or 1% or less of the  
polypeptide sequence.

15 Another example of a conservatively modified variation (e.g., a  
“conservatively substituted variation”) of the polypeptide identified herein as SEQ ID NO:  
75 will contain “conservative substitutions” according to the six substitution groups set forth  
in Table 2 (*supra*), in up to about 9 residues (i.e., less than about 5%) of the 180 amino acid  
polypeptide.

20 The lipase polypeptide sequence homologues of the invention, including  
conservatively substituted sequences, can be present as part of larger polypeptide sequences  
such as occur upon the addition of one or more domains for purification of the protein (e.g.,  
poly his segments, FLAG tag segments, etc.), e.g., where the additional functional domains  
have little or no effect on the activity of the lipase portion of the protein, or where the  
25 additional domains can be removed by post synthesis processing steps such as by treatment  
with a protease.

In various embodiments, the polypeptide comprises at least about 45, 50, 75,  
100, 125, 150, 175, 200 or at least about 215, or more contiguous amino acid residues of any  
of SEQ ID NO: 55 to SEQ ID NO: 108. Alternatively, the polypeptide comprises at least  
30 about 180 contiguous amino acids residues, at least about 212 contiguous amino acid

residues, at least about 213 contiguous amino acid residues, or at least about 215 amino acid residues of any of SEQ ID NO: 55 to SEQ ID NO: 108.

#### DEFINING POLYPEPTIDES BY IMMUNOREACTIVITY

Because the polypeptides of the invention provide a variety of new polypeptide sequences as compared to other lipases, the polypeptides also provide new structural features which can be recognized, e.g., in immunological assays. The generation of antisera which specifically binds the polypeptides of the invention, as well as the polypeptides which are bound by such antisera, are a feature of the invention.

The invention includes lipase homologue proteins that specifically bind to or that are specifically immunoreactive with an antibody or antisera generated against an immunogen comprising an amino acid sequence selected from one or more of SEQ ID NO: 55 to SEQ ID NO: 108. To eliminate cross-reactivity with other lipases, the antibody or antisera is subtracted with available homologues such as those found in GenBank represented by or encoded by GenBank accession numbers 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108 (i.e., the "control" lipase homologue polypeptides). Proteins that can bind specifically as described above can be determined by aligning any of SEQ ID NO: 55 to SEQ ID NO: 108 against the complete set of nucleic acids corresponding or encoded by: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108. Where the GenBank sequence corresponds to a nucleic acid, a polypeptide encoded by the nucleic acid is generated and used for antibody/antisera subtraction purposes. Where the nucleic acid corresponds to a non-coding sequence, e.g., a pseudo gene, an amino acid which corresponds

to the reading frame of the nucleic acid is generated (e.g., synthetically), or is minimally modified to include a start codon for recombinant production.

In one typical format, the immunoassay uses a polyclonal antiserum which was raised against one or more polypeptide comprising one or more of the sequences  
5 corresponding to one or more of SEQ ID NO: 55 to SEQ ID NO: 108, or a substantial subsequence thereof (i.e., at least about 30% of the full length sequence provided). The full set of potential polypeptide immunogens derived from SEQ ID NO: 55 to SEQ ID NO:108 are collectively referred to below as "the immunogenic polypeptides." The resulting antisera is optionally selected to have low cross-reactivity against the control lipase homologues and  
10 any other known homologues and any such cross-reactivity is removed by immunoabsorbtion with one or more of the control lipase homologues, or other known homologues, prior to use of the polyclonal antiserum in the immunoassay.

In order to produce antisera for use in an immunoassay, one or more of the immunogenic polypeptides is produced and purified as described herein. For example,  
15 recombinant protein may be produced in a mammalian cell line. An inbred strain of mice (used in this assay because results are more reproducible due to the virtual genetic identity of the mice) is immunized with the immunogenic protein(s) in combination with a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (*see*, Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor  
20 Publications, New York, for a standard description of antibody generation, immunoassay formats and conditions that can be used to determine specific immunoreactivity). Alternatively, one or more synthetic or recombinant polypeptide derived from the sequences disclosed herein is conjugated to a carrier protein and used as an immunogen.

Polyclonal sera are collected and titered against the immunogenic polypeptide  
25 in an immunoassay, for example, a solid phase immunoassay with one or more of the immunogenic proteins immobilized on a solid support. Polyclonal antisera with a titer of  $10^6$  or greater are selected, pooled and subtracted with the control lipase homologue polypeptides to produce subtracted pooled titered polyclonal antisera.

The subtracted pooled titered polyclonal antisera are tested for cross reactivity  
30 against the control lipase homologues (e.g., as enumerated herein). Preferably at least two of

the immunogenic lipase homologues are used in this determination, preferably in conjunction with at least two of the control lipase homologues, to identify antibodies which are specifically bound by the immunogenic protein(s).

In this comparative assay, discriminatory binding conditions are determined  
5 for the subtracted titered polyclonal antisera which result in at least about a 5-10 fold higher signal to noise ratio for binding of the titered polyclonal antisera to the immunogenic lipase molecules as compared to binding to any control homologues. That is, the stringency of the binding reaction is adjusted by the addition of non-specific competitors such as albumin or non-fat dry milk, or by adjusting salt conditions, temperature, or the like. These binding  
10 conditions are used in subsequent assays for determining whether a test polypeptide is specifically bound by the pooled subtracted polyclonal antisera. In particular, test polypeptides which show at least a 2-5x higher signal to noise ratio than the control polypeptides under discriminatory binding conditions, and at least about a ½ signal to noise ratio as compared to the immunogenic polypeptide(s), shares substantial structural similarity  
15 with the immunogenic polypeptide as compared to control polypeptides, and is, therefore a polypeptide of the invention.

In another example, immunoassays in the competitive binding format are used for detection of a test polypeptide. For example, as noted, cross-reacting antibodies are removed from the pooled antisera mixture by immunoabsorbtion with the control lipase  
20 polypeptides. The immunogenic lipase homologue polypeptide(s) are then immobilized to a solid support which is exposed to the subtracted pooled antisera. Test proteins are added to the assay to compete for binding to the pooled subtracted antisera. The ability of the test protein(s) to compete for binding to the pooled subtracted antisera as compared to the immobilized protein(s) is compared to the ability of the immunogenic polypeptide(s) added  
25 to the assay to compete for binding (the immunogenic polypeptides compete effectively with the immobilized immunogenic polypeptides for binding to the pooled antisera). The percent cross-reactivity for the test proteins is calculated, using standard calculations.

In a parallel assay, the ability of the control proteins to compete for binding to the pooled subtracted antisera is determined as compared to the ability of the immunogenic  
30 polypeptide(s) to compete for binding to the antisera. Again, the percent cross-reactivity for

the control polypeptides is calculated, using standard calculations. Where the percent cross-reactivity is at least 5-10x as high for the test polypeptides, the test polypeptides are said to specifically bind the pooled subtracted antisera.

In general, the immunoabsorbed and pooled antisera can be used in a competitive binding immunoassay as described herein to compare any test polypeptide to the immunogenic polypeptide(s). In order to make this comparison, the two polypeptides are each assayed at a wide range of concentrations and the amount of each polypeptide required to inhibit 50% of the binding of the subtracted antisera to the immobilized protein is determined using standard techniques. If the amount of the test polypeptide required is less than twice the amount of the immunogenic polypeptide that is required, then the test polypeptide is said to specifically bind to an antibody generated to the immunogenic protein, provided the amount is at least about 5-10x as high as for a control polypeptide.

As a final determination of specificity, the pooled antisera is optionally fully immunosorbed with the *immunogenic* polypeptide(s) (rather than any control polypeptides) until little or no binding of the resulting immunogenic polypeptide subtracted pooled antisera to the immunogenic polypeptide(s) used in the immunosorbition is detectable. This fully immunosorbed antisera is then tested for reactivity with the test polypeptide. If little or no reactivity is observed (i.e., no more than 2x the signal to noise ratio observed for binding of the fully immunosorbed antisera to the immunogenic polypeptide), then the test polypeptide is specifically bound by the antisera elicited by the immunogenic protein.

#### ENANTIOSELECTIVE LIPASE ACTIVITY

As described previously, enantiomers are non-superimposable stereoisomers of a molecule. In other words, they are mirror images of each other. Enantiomers of a molecule have identical melting points, boiling points, densities, refractive indexes, etc. However one form rotates plane-polarized light to the right while the other enantiomer rotates it to the left. In fact, enantiomers are often designated as (+) or (-) forms of the molecule. Alternatively, the forms can be labeled as *cis* and *trans* forms of the molecule.

Even though enantiomers share many identical properties, when they interact with other molecules that are also stereochemically specific, differing results (e.g., products) can result, depending upon which form (*cis* or *trans*) interacts with the other molecule. Most

enzymes and many other molecules in biological systems are stereochemically specific. Thus, the proper enantiomeric form of a molecule can be important if a desired result is to be achieved. This is true both in biological/pharmacological situations as well as in industrial settings.

5                   For example (+) glucose is a commonly metabolized sugar and is extremely important in, e.g., industrial yeast fermentation. However, (-) glucose (i.e., the opposite enantiomeric form of glucose) is not commonly metabolized in animals or yeast, etc. Numerous other examples of such differences exist, such as: (+) glutamic acid/(-) glutamic acid (only one is used as a flavor enhancer); (+) carvone/(-) carvone (one smells of spearmint  
10 while the other smells of caraway); and (+) chloromycetin/(-) chloromycetin (only one has antibiotic properties), etc.

                  Not only can opposing enantiomers be selectively useful or have different uses, but in some situations one enantiomer can interfere with the usage of its opposing form. For example, (+) ephedrine has no drug activity and also interferes with the action of its  
15 opposing enantiomer (i.e., (-) ephedrine).

                  Thus, enzymes specific for interaction with a specific enantiomeric form of a substrate would be extremely useful in a myriad of chemical/industrial and clinical settings. For example, a degradative enzyme that was enantioselective for (+) ephedrine could be used to aid in purification of (-) ephedrine from a mixed population (racemic) of the 2  
20 enantiomers.

                  The lipase homologue polypeptides of the current invention were screened for enantioselective lipase activity on neryl butyrate and geranyl butyrate. Again, while the current assays screened with respect to neryl/geranyl butyrate (*see, EXAMPLE II*), it will be appreciated that the lipase homologues of the invention optionally display lipase and/or  
25 enantioselective lipase activity with respect to a number of different substrates (e.g., neryl/geranyl acetate, tributyrin, methyl esters, etc.). Geranyl butyrate is the *trans* isomer of 3,7-dimethyl-2,6-octadien-1-yl butyrate while neryl-butyrate is the *cis* isomer of the same compound. Both neryl and geranyl butyrate have industrial uses, e.g., as precursors, etc. in the perfume/fragrance industry.

The enantioselectivity of the lipase homologue polypeptides of the invention was determined by measuring the enantiomeric ratio or "E." The enantiomeric ratio is determined by the equation:

$$5 \quad E = \frac{\ln [ 1 - c (1 + DE(p)) ]}{\ln [ 1 - c (1 - DE(p)) ]}$$

in which c = the percent total substrate conversion (expressed as a decimal) and DE(p) is the diastereomeric excess (i.e., the percent product of a first isomer minus the percent product of a second isomer) of the products.

10 Figure 1 shows the enantioselectivity of the newly created lipase homologue polypeptides of the invention for neryl and geranyl butyrate. As can be seen, specific clones created had specificity for either neryl butyrate or geranyl butyrate.

In other aspects, such isolated or recombinant polypeptide comprises an amino acid sequence of any one of SEQ ID NO: 55 through SEQ ID NO: 108 over a  
15 comparison window of at least 45 contiguous amino acids.

In some embodiments, the invention comprises such an isolated or recombinant polypeptide that is at least 45 contiguous amino acid residues of a polypeptide encoded by a coding polynucleotide sequence wherein the polynucleotide sequence is selected from: a polynucleotide sequence from any of SEQ ID NO: 1 to SEQ ID NO: 54, a  
20 polynucleotide sequence that encodes a polypeptide selected from any of SEQ ID NO: 55 through SEQ ID NO: 108; or a polynucleotide sequence that hybridizes under stringent conditions over substantially the entire length of the above polynucleotide sequence or which hybridizes to a subsequence comprising at least about 100 nucleic acids, provided that none of the sequences corresponds to or encodes any of GenBank accession numbers: 1I6WA,  
25 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105,  
30 and Z99108.



Additionally, the invention provides such isolated or recombinant polypeptide wherein the polypeptide is enantioselective for either a *cis* form substrate enantiomer or for a *trans* form substrate enantiomer and optionally wherein the enantiomeric ratio is at least 2 or more, at least 5 or more, at least 10 or more, at least 50 or more, or at least 100 or more.

5           The invention also provides such isolated or recombinant polypeptide wherein the identity is determined by a sequence alignment performed using BLASTP with default parameters set to measure a desired identity (*see above*). Additionally, which polypeptide comprises an amino acid sequence of any of SEQ ID NO: 55 through SEQ ID NO: 108 and/or wherein the identity is determined by a sequence alignment using BLASTP with  
10 default parameters set to measure a desired identity.

          Additionally the invention comprises an isolated or recombinant polypeptide that is at least 90, at least 94, at least 95, at least 96, at least 97, at least 98, at least 99 or more percent identical over a comparison window of 45 contiguous amino acids (or 50, 75, 100, 125, 150, 175, 200, 180, 212, 213, or 215 contiguous amino acids) of one or more of SEQ ID  
15 NO: 55 through SEQ ID NO: 108. Also, the invention provides an isolated or recombinant polypeptide identified by performing a sequence alignment with any one or more of SEQ ID NO: 55 through SEQ ID NO: 108 using BLASTP with default parameters set to measure a desired identity.

#### COMMERCIAL/INDUSTRIAL METHODS AND COMPOSITIONS

20           The lipase homologues of the invention are optionally used in compositions to accomplish numerous commercial and industrial procedures. The lipases of the invention are optionally used in the synthesis and/or degradation of specific lipids (*i.e.*, to break down longer lipids and thus synthesize more desirable lipid molecules).

          Other non-limiting examples of commercial/industrial uses of the current.  
25 lipase homologues include: use as supplements in animal feeds, as agents of flavor modification and fat modification in human foodstuffs (*e.g.*, cheese), as agents in the creation of food emulsifiers such as distilled monoglyceride, as agents in the production of fatty acid esters for texturing agents (*e.g.*, for use in cosmetics), as aids in fractionation of fats, as means to remove unwanted types of lipids from lipid mixtures thus effectively concentrating  
30 the remaining lipid types (*e.g.*, as a means to increase the percentage of "healthful" fish oils

in mixtures such as dietary supplements), as agents in tanning/processing leather, and as cleaning agents (*see*, below).

The lipases of the invention are also optionally immobilized on substrates, e.g., cellulose fibers, capillary tubes, various microchip structures, etc. during use, thus, optionally permitting increased reaction periods, multiple reuse of the lipase molecules, avoidance of the need to purify out lipase molecules once they are no longer needed, etc.

### CLEANING SOLUTIONS

The lipase homologues of the invention are favorably used in compositions that serve as cleaning solutions in wide variety of applications, including laundry detergents, contact lens cleansing solutions, and dry cleaning, among others.

For example, the present invention provides the use of the novel lipase homologues of the invention in cleaning and detergent compositions, as well as such compositions containing mutant lipase enzymes. Such cleaning and detergent compositions can in principle have any physical form, but the lipase homologues are preferably incorporated in liquid detergent compositions or in detergent compositions in the form of bars, tablets, sticks and the like for direct application, wherein they exhibit improved enzyme stability or performance.

Among the liquid compositions of the present invention are aqueous liquid detergents having for example a homogeneous physical character, e.g. they can consist of a micellar solution of surfactants in a continuous aqueous phase, so-called isotropic liquids. Alternatively, they can have a heterogeneous physical phase and they can be structured, containing suspended solid particles such as particles of builder materials e.g. of the kinds mentioned below. In addition, the liquid detergents according to the present invention can include an enzyme stabilization system, comprising calcium ion, boric acid, propylene glycol and/or short chain carboxylic acids. Optionally, the detergents include additional enzyme components including, e.g., cellulase, amylase, subtilisin, or proteases.

In addition, powder detergent compositions can include, in addition to any one or more of the lipase homologues of the invention as described herein, such components as builders (such as phosphate or zeolite builders), surfactants (such as anionic, cationic, non-ionic or zwitterionic type surfactants), polymers (such as acrylic or equivalent polymers),

bleach systems (such as perborate- or amino-containing bleach precursors or activators),  
structurants (such as silicate structurants), alkali or acid to adjust pH (i.e., a pH adjuster),  
humectants, and/or neutral inorganic salts. Furthermore, a number of other ingredients are  
normally present in the compositions of the invention, such as co-surfactants, tartrate  
5 succinate builder, neutralization system, suds suppressor, other enzymes and other optional  
components.

#### THERAPEUTIC AND PROPHYLACTIC METHODS AND COMPOSITIONS

Lipases, including the lipase homologue polypeptides and their encoding  
nucleic acids, are optionally used in the therapeutic and/or prophylactic treatment of a  
10 number of medical diseases/disorders/conditions.

For example, lipase treatment of subjects is optionally useful in conditions  
such as, but not limited to: Crohn's disease, cystic fibrosis, celiac disease, pancreatic  
abnormalities (e.g., chronic pancreatitis), nonspecific indigestion, and other gastrointestinal  
mal-absorption problems.

15 The amount of lipase polypeptide given in current treatments of such  
conditions is variable (as is the normal level of intrinsic lipase) and is preferably adjusted by  
a physician to a subject's specific medical condition. In some clinical situations lipase  
supplements are given in combination with supplements of other enzymes (e.g., amylases,  
proteolytic enzymes, etc.) to help in treatment. As detailed below, the nucleic acids of the  
20 current invention are also optionally utilized in treatment of medical conditions.

The present invention also includes methods of therapeutically or  
prophylactically treating a disease or disorder by administering, in vivo or ex vivo, one or  
more nucleic acids or fragments thereof or polypeptides or fragments thereof of the invention  
described above (or compositions comprising a pharmaceutically acceptable excipient and  
25 one or more such nucleic acids or polypeptides) to a subject, including, e.g., a mammal,  
including, e.g.; a human, primate, mouse, pig, cow, goat, rabbit, rat, guinea pig, hamster,  
horse, sheep; or a non-mammalian vertebrate such as a bird (e.g., a chicken or duck) or a fish,  
or invertebrate.

In one aspect of the invention, in ex vivo methods, one or more cells, or a  
30 population of cells of interest of the subject (e.g., tumor cells, tumor tissue sample, organ

cells, blood cells, cells of the skin, lung, heart, muscle, brain, mucosae, liver, intestine, spleen, stomach, lymphatic system, cervix, vagina, prostate, mouth, tongue, etc.) are obtained or removed from the subject and contacted with an amount of a polypeptide of the invention that is effective in prophylactically or therapeutically treating a disease, disorder, or other  
5 condition. The contacted cells are then returned or delivered to the subject to the site from which they were obtained or to another site (e.g., including those defined above) of interest in the subject to be treated. If desired, the contacted cells may be grafted onto a tissue, organ, or system site (including all described above) of interest in the subject using standard and well-known grafting techniques or, e.g., delivered to the blood or lymph system using  
10 standard delivery or transfusion techniques.

The invention also provides *in vivo* methods in which one or more cells or a population of cells of interest of the subject are contacted directly or indirectly with an amount of a polypeptide of the invention effective in prophylactically or therapeutically treating a disease, disorder, or other condition. In direct contact/administration formats, the  
15 polypeptide is typically administered or transferred directly to the cells to be treated or to the tissue site of interest (e.g., tumor cells, tumor tissue sample, organ cells, blood cells, cells of the skin, lung, heart, muscle, brain, mucosae, liver, intestine, spleen, stomach, lymphatic system, cervix, vagina, prostate, mouth, tongue, etc.) by any of a variety of formats, including topical administration, injection (e.g., by using a needle and/or syringe), or vaccine  
20 or gene gun delivery, pushing into a tissue, organ, or skin site. The polypeptide can be delivered, for example, intramuscularly, intradermally, subdermally, subcutaneously, orally, intraperitoneally, intrathecally, intravenously, or placed within a cavity of the body (including, e.g., during surgery), or by inhalation or vaginal or rectal administration.

In *in vivo* indirect contact/administration formats, the polypeptide is typically  
25 administered or transferred indirectly to the cells to be treated or to the tissue site of interest, including those described above (such as, e.g., skin cells, organ systems, lymphatic system, or blood cell system, etc.), by contacting or administering the polypeptide of the invention directly to one or more cells or population of cells from which treatment can be facilitated. For example, specific cells (e.g., tumor cells) within the body of the subject can be treated by  
30 contacting cells of the blood or lymphatic system, skin, or an organ with a sufficient amount

of the polypeptide such that delivery of the polypeptide to the site of interest (e.g., tissue, organ, or cells of interest or blood or lymphatic system within the body) occurs and effective prophylactic or therapeutic treatment results. Such contact, administration, or transfer is typically made by using one or more of the routes or modes of administration described  
5 above.

In another aspect, the invention provides ex vivo methods in which one or more cells of interest or a population of cells of interest of the subject (e.g., tumor cells, tumor tissue sample, organ cells, blood cells, cells of the skin, lung, heart, muscle, brain, mucosae, liver, intestine, spleen, stomach, lymphatic system, cervix, vagina, prostate, mouth,  
10 tongue, etc.) are obtained or removed from the subject and transformed by contacting said one or more cells or population of cells with a polynucleotide construct comprising a target nucleic acid sequence of the invention or fragments thereof, that encodes a biologically active polypeptide of interest (e.g., a polypeptide of the invention) that is effective in prophylactically and/or therapeutically treating the disease, disorder, or other condition. The  
15 one or more cells or population of cells is contacted with a sufficient amount of the polynucleotide construct and a promoter controlling expression of said nucleic acid sequence such that uptake of the polynucleotide construct (and promoter) into the cell(s) occurs and sufficient expression of the target nucleic acid sequence of the invention results to produce an amount of the biologically active polypeptide effective to prophylactically and/or  
20 therapeutically treat the disease, disorder, or condition. The polynucleotide construct may include a promoter sequence (e.g., CMV promoter sequence) that controls expression of the nucleic acid sequence of the invention and/or, if desired, one or more additional nucleotide sequences encoding at least one or more of another polypeptide of the invention, a cytokine, adjuvant, or co-stimulatory molecule, or other polypeptide of interest.

Following transfection, the transformed cells are returned, delivered, or transferred to the subject to the tissue site or system from which they were obtained or to another site (e.g., tumor cells, tumor tissue sample, organ cells, blood cells, cells of the skin, lung, heart, muscle, brain, mucosae, liver, intestine, spleen, stomach, lymphatic system, cervix, vagina, prostate, mouth, tongue, etc.) to be treated in the subject. If desired, the cells  
30 may be grafted onto a tissue, skin, organ, or body system of interest in the subject using

standard and well-known grafting techniques or delivered to the blood or lymphatic system using standard delivery or transfusion techniques. Such delivery, administration, or transfer of transformed cells is typically made by using one or more of the routes or modes of administration described above. Expression of the target nucleic acid occurs naturally or can  
5 be induced (as described in greater detail below) and an amount of the encoded polypeptide is expressed sufficient and effective to treat the disease or condition at the site or tissue system (or at another site within the subject).

In another aspect, the invention provides in vivo methods in which one or more cells of interest or a population of cells of the subject (e.g., including those cells and  
10 cell(s) systems and subjects described above) are transformed in the body of the subject by contacting the cell(s) or population of cells with (or administering or transferring to the cell(s) or population of cells using one or more of the routes or modes of administration described above) a polynucleotide construct comprising a nucleic acid sequence of the invention that encodes a biologically active polypeptide of interest (e.g., a polypeptide of the  
15 invention) that is effective in prophylactically and/or therapeutically treating the disease, disorder, or other condition.

The polynucleotide construct can be directly administered or transferred to cell(s) suffering from the disease or disorder (e.g., by direct contact using one or more of the routes or modes of administration described above). Alternatively, the polynucleotide  
20 construct can be indirectly administered or transferred to cell(s) suffering from the disease or disorder by first directly contacting non-diseased cell(s) or other diseased cells using one or more of the routes or modes of administration described above with a sufficient amount of the polynucleotide construct comprising the nucleic acid sequence encoding the biologically active polypeptide, and a promoter controlling expression of the nucleic acid sequence, such  
25 that uptake of the polynucleotide construct (and promoter) into the cell(s) occurs and sufficient expression of the nucleic acid sequence of the invention results to produce an amount of the biologically active polypeptide effective to prophylactically and/or therapeutically treat the disease or disorder, and whereby the polynucleotide construct or the resulting expressed polypeptide is transferred naturally or automatically from the initial  
30 delivery site, system, tissue or organ of the subject's body to the diseased site, tissue, organ

or system of the subject's body (e.g., via the blood or lymphatic system). Expression of the target nucleic acid occurs naturally or can be induced (as described in greater detail below) such that an amount of the encoded polypeptide expressed is sufficient and effective to treat the disease or condition at the site or tissue system. The polynucleotide construct may  
5 include a promoter sequence (e.g., CMV promoter sequence) that controls expression of the nucleic acid sequence and/or, if desired, one or more additional nucleotide sequences encoding at least one or more of another polypeptide of the invention, a cytokine, adjuvant, or co-stimulatory molecule, or other polypeptide of interest.

In each of the in vivo and ex vivo treatment methods as described above, a  
10 composition comprising an excipient and the polypeptide or nucleic acid of the invention can be administered or delivered. In one aspect, a composition comprising a pharmaceutically acceptable excipient and a polypeptide or nucleic acid of the invention is administered or delivered to the subject as described above in an amount effective to treat the disease or disorder.

In another aspect, in each in vivo and ex vivo treatment method described  
15 above, the amount of polynucleotide administered to the cell(s) or subject can be an amount sufficient that uptake of said polynucleotide into one or more cells of the subject occurs and sufficient expression of said nucleic acid sequence results to produce an amount of a biologically active polypeptide effective to enhance an immune response in the subject,  
20 including an immune response induced by an immunogen (e.g., antigen). In another aspect, for each such method, the amount of polypeptide administered to cell(s) or subject can be an amount sufficient to enhance an immune response in the subject, including that induced by an immunogen (e.g., antigen).

In yet another aspect, in each in vivo and ex vivo treatment method described  
25 above, the amount of polynucleotide administered to the cell(s) or subject can be an amount sufficient that uptake of said polynucleotide into one or more cells of the subject occurs and sufficient expression of said nucleic acid sequence results to produce an amount of a biologically active polypeptide effective to produce a tolerance or anergy response in the subject. In another aspect, for each such method, the amount of polypeptide administered to

cell(s) or subject can be an amount sufficient to produce a tolerance or anergy response in the subject.

In yet another aspect, in an *in vivo* or *in vivo* treatment method in which a polynucleotide construct (or composition comprising a polynucleotide construct) is used to deliver a physiologically active polypeptide to a subject, the expression of the polynucleotide construct can be induced by using an inducible on- and off-gene expression system. Examples of such on- and off-gene expression systems include the Tet-On™ Gene Expression System and Tet-Off™ Gene Expression System (*see, e.g.,* Clontech Catalog 2000, pg. 110-111 for a detailed description of each such system), respectively. Other controllable or inducible on- and off-gene expression systems are known to those of ordinary skill in the art. With such system, expression of the target nucleic of the polynucleotide construct can be regulated in a precise, reversible, and quantitative manner. Gene expression of the target nucleic acid can be induced, for example, after the stable transfected cells containing the polynucleotide construct comprising the target nucleic acid are delivered or transferred to or made to contact the tissue site, organ or system of interest. Such systems are of particular benefit in treatment methods and formats in which it is advantageous to delay or precisely control expression of the target nucleic acid (e.g., to allow time for completion of surgery and/or healing following surgery; to allow time for the polynucleotide construct comprising the target nucleic acid to reach the site, cells, system, or tissue to be treated; to allow time for the graft containing cells transformed with the construct to become incorporated into the tissue or organ onto or into which it has been spliced or attached, etc.).

Therapeutic compositions comprising one or more lipase homologue polypeptide of the invention are tested in appropriate *in vitro* and *in vivo* animal models of disease, to confirm efficacy, tissue metabolism, and to estimate dosages, according to methods well known in the art.

Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood or tissue cells. The lipase homologues of the invention are administered in any suitable manner, preferably with pharmaceutically acceptable carriers. Suitable methods of administering such lipase homologues in the context of the present invention to a patient are available, and, although more than one route can be



used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions of the present invention.

Polypeptide compositions can be administered by a number of routes including, but not limited to oral, intravenous, intraperitoneal, intramuscular, transdermal, subcutaneous, topical, sublingual, or rectal means. Lipase homologue polypeptide compositions can also be administered via liposomes. Such administration routes and appropriate formulations are generally known to those of skill in the art.

The lipase homologue, alone or in combination with other suitable components, can also be made into aerosol formulations (i.e., they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations of packaged nucleic acid can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

Parenteral administration and intravenous administration are preferred methods of administration. In particular, the routes of administration already in use for lipase related therapeutic agents, along with formulations in current use, are preferred routes of administration and formulation for the lipase polypeptides of the invention.

Cells transduced with the lipase homologue nucleic acids as described above in the context of ex vivo therapy can also be administered intravenously or parenterally as

described above. It will be appreciated that the delivery of cells to patients is routine, e.g., delivery of cells to the blood via intravenous or intraperitoneal administration.

The dose administered to a patient, in the context of the present invention is sufficient to effect a beneficial therapeutic response in the patient over time, depending on the application. The dose will be determined by the efficacy of the particular vector, or  
5 formulation, and the activity lipase homologue employed and the condition of the patient, as well as the body weight or surface area of the patient to be treated. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular vector, formulation, transduced cell type or the  
10 like in a particular patient.

In determining the effective amount of the vector, cell type, or formulation to be administered in the treatment or prophylaxis of a disease/condition/etc., the physician evaluates circulating plasma levels, vector/cell/formulation/ lipase homologue toxicities, progression of the disease, and the production of anti-vector/ lipase homologue antibodies.

The dose administered, e.g., to a 70 kilogram patient will be in the range equivalent to dosages of currently-used lipase related therapeutic proteins, and doses of vectors or cells which produce lipase homologue sequences are calculated to yield an equivalent amount of lipase homologue nucleic acid or expressed protein. The vectors of this invention can supplement the treatment of cancers and virally-mediated conditions by any  
15 known conventional therapy, including cytotoxic agents, nucleotide analogues (e.g., when used for treatment of HIV infection), biologic response modifiers, and the like.

For administration, lipase homologues and transduced cells of the present invention can be administered at a rate determined by the LD-50 of the lipase homologue, vector, or transduced cell type, and the side-effects of the lipase homologues, vector or cell  
25 type at various concentrations, as applied to the mass and overall health of the patient. Administration can be accomplished via single or divided doses.

For example, in the therapeutic and prophylactic treatment methods of the invention described herein, an effective amount of a lipase nucleic acid (e.g., DNA or mRNA) of the invention (e.g., nucleic acid dosage) will generally be in the range of, e.g.,  
30 from about 0.05 microgram/kilogram (kg) to about 50 mg/kg, usually about 0.005-5 mg/kg.

However, as will be understood, the effective amount of the nucleic acid (e.g., nucleic acid dosage) and/or polypeptide (e.g., polypeptide dosage) will vary in a manner apparent to those of ordinary skill in the art according to a number of factors, including the activity or potency of the polypeptide, the activity or potency of any nucleic acid construct (e.g., vector, promoter, expression system) to be administered, the disease or condition to be treated, and the subject to which or whom the nucleic acid is delivered.

For delivery of some polypeptides, e.g., by delivering nucleic acids encoding such polypeptides, for example, adequate levels of translation and/or expression are achieved with a nucleic acid dosage of, e.g., about 0.005mg/kg to about 5 mg/kg. Dosages for other polypeptides (and nucleic acids encoding them) having a known biological activity can be readily determined by those of skill in the art according to the factors noted above. Dosages used for other known lipase related nucleic acids and polypeptides for particular diseases provide guidelines for determining dosage and treatment regimen for a nucleic acid or polypeptide of the invention. An effective amount of a lipase homologue polypeptide may be in the range of from about 1 microgram to about 1 milligram, and more typically from about 1 microgram to about 100 micrograms.

A composition for use in therapeutic and prophylactic treatment methods of the invention described herein may comprise, e.g., a concentration of a lipase homologue nucleic acid (e.g., DNA or mRNA) of the invention of from about 0.1 microgram/milliliter (ml) to about 20 mg/ml and a pharmaceutically acceptable carrier (e.g., aqueous carrier).

A composition for use in therapeutic and/or prophylactic treatment methods of the invention described herein may comprise, e.g., a concentration of a lipase homologue polypeptide of the invention in an amount as described above and herein and a pharmaceutically acceptable carrier (e.g., aqueous carrier).

For introduction of recombinant lipase nucleic acid transduced cells into a patient, blood samples are obtained prior to infusion, and saved for analysis. Between  $1 \times 10^6$  and  $1 \times 10^{12}$  transduced cells are infused intravenously over 60- 200 minutes. Vital signs and oxygen saturation by pulse oximetry are closely monitored. Blood samples are obtained 5 minutes and 1 hour following infusion and saved for subsequent analysis. Leukopheresis, transduction and reinfusion are optionally repeated every 2 to 3 months for a

total of 4 to 6 treatments in a one year period. After the first treatment, infusions can be performed on a outpatient basis at the discretion of the clinician. If the reinfusion is given as an outpatient, the participant is monitored for at least 4, and preferably 8 hours following the therapy. Transduced cells are prepared for reinfusion according to established methods. See, 5 Abrahamsen et al. (1991) J Clin Apheresis 6:48-53; Carter et al. (1988) J Clin Apheresis 4:113-117; Aebersold et al. (1988), J Immunol Methods 112: 1-7; Muul et al. (1987) J Immunol Methods 101:171-181 and Carter et al. (1987) Transfusion 27:362-365. After a period of about 2-4 weeks in culture, the cells should number between  $1 \times 10^6$  and  $1 \times 10^{12}$ . In this regard, the growth characteristics of cells vary from patient to patient and from cell 10 type to cell type. About 72 hours prior to reinfusion of the transduced cells, an aliquot is taken for analysis of phenotype, and percentage of cells expressing the therapeutic agent.

If a patient undergoing infusion of a vector or transduced cell or protein formulation develops fevers, chills, or muscle aches, he/she receives the appropriate dose of aspirin, ibuprofen, acetaminophen or other pain/fever controlling drug. Patients who 15 experience reactions to the infusion such as fever, muscle aches, and chills are premedicated 30 minutes prior to the future infusions with either aspirin, acetaminophen, or, e.g., diphenhydramine. Meperidine is used for more severe chills and muscle aches that do not quickly respond to antipyretics and antihistamines. Cell infusion is slowed or discontinued depending upon the severity of the reaction.

20 The current invention provides methods to therapeutically or prophylactically treat a gastrointestinal lipid related condition/disease/disorder by hydrolyzing a lipid through expressing in a target cell, or contacting a target cell, with an effective amount of polypeptide of the invention (or a fragment thereof) both wherein such target cell is in culture and wherein such target cell is within a subject to be treated. The current invention also provides 25 a method of therapeutic or prophylactic treatment of a gastrointestinal lipid related condition/disease/disorder in a subject wherein the subject is administered a polypeptide of the invention in an amount effect to treat the condition/disease/disorder, including wherein the subject is a mammal or more specifically, a human, and wherein the polypeptide is administered in vivo, in vitro, or ex vivo (or a combination of such) to one or more cells of 30 the subject. Such polypeptides include compositions of polypeptides comprising the

polypeptide and a pharmaceutically acceptable excipient, which is administered to a subject in an amount effective to treat a gastrointestinal lipid related condition/disease/disorder (e.g., cystic fibrosis, celiac disease, Crohn's disease, indigestion, and obesity)

Another provision of the invention is a method of hydrolyzing a lipid to  
5 therapeutically or prophylactically treat a gastrointestinal lipid related  
condition/disease/disorder by introducing into a target cell a nucleic acid of the invention, or a fragment thereof, which is operably linked to a regulatory sequence active in a target cell such that introduction of the polynucleotide results in expression of the nucleic acid in an amount sufficient to hydrolyze the lipid. Such method optionally comprises directly  
10 administering the nucleic acid to a subject in an amount sufficient to introduce the nucleic acid into one or more cells and wherein the subject comprises a mammal (or a human) and wherein the nucleic acid optionally comprises a vector. Yet another provision of the invention is a method of therapeutically or prophylactically treating a gastrointestinal lipid related condition/disease/disorder by expressing in a target cell (or contacting a target cell  
15 with an effective amount of) a polynucleotide of the invention, or a fragment thereof, or of a polypeptide encoded thereby (or a fragment thereof). Such method can comprise wherein the target is in culture or wherein the target cell is within a subject. Additionally, the invention provides a method of therapeutically or prophylactically treating a gastrointestinal lipid related condition/disease/disorder in a subject by administering to the subject a  
20 polynucleotide of the invention (or a fragment thereof) or a polypeptide encoded thereby (or a fragment thereof) in an amount effective to treat the gastrointestinal lipid related condition/disease/disorder. Such method comprises optional embodiments wherein the subject is a mammal or a human and wherein the polynucleotide and/or polypeptide is administered in vivo, in vitro, or ex vivo (or a combination of such) to one or more cells of  
25 the subject and wherein a composition of the polynucleotide and/or polypeptide and a pharmaceutically acceptable excipient is administered to the subject in an amount effective to treat the gastrointestinal lipid related condition/disease/disorder (e.g., cystic fibrosis, celiac disease, Crohn's disease, indigestion, or obesity).

### INTEGRATED SYSTEMS

The present invention provides computers, computer readable media and integrated systems comprising character strings corresponding to the sequence information herein for the polypeptides and nucleic acids herein, including, e.g., those sequences listed  
5 herein and the various silent substitutions and conservative substitutions thereof.

Various methods and genetic algorithms (GAs) known in the art can be used to detect homology or similarity between different character strings, or can be used to perform other desirable functions such as to control output files, provide the basis for making presentations of information including the sequences and the like. Examples include  
10 BLAST, discussed *supra*. Extensive examples of the use of sequences in silico are found in, e.g., PCT/US00/01202 "METHODS FOR MAKING CHARACTER STRINGS, POLYNUCLEOTIDES AND POLYPEPTIDES HAVING DESIRED CHARACTERISTICS" by Selifonov et al., filed Jan. 18, 2000; PCT/US00/01230 "OLIGONUCLEOTIDE MEDIATED NUCLEIC ACID RECOMBINATION" by Crameri et  
15 al., filed Jan. 18, 2000; and PCT/US00/01138 "METHODS OF POPULATING DATA STRUCTURES FOR USE IN EVOLUTIONARY SIMULATIONS" by Selifonov and Stemmer, filed Jan. 18, 2000.

Thus, different types of homology and similarity of various stringency and length can be detected and recognized in the integrated systems herein. For example, many  
20 homology determination methods have been designed for comparative analysis of sequences of biopolymers, for spell-checking in word processing, and for data retrieval from various databases. With an understanding of double-helix pair-wise complement interactions among 4 principal nucleobases in natural polynucleotides, models that simulate annealing of complementary homologous polynucleotide strings can also be used as a foundation of  
25 sequence alignment or other operations typically performed on the character strings corresponding to the sequences herein (e.g., word-processing manipulations, construction of figure comprising sequence or subsequence character strings, output tables, etc.). An example of a software package with GAs for calculating sequence similarity is BLAST, which can be adapted to the present invention by inputting character strings corresponding to  
30 the sequences herein.

Similarly, standard desktop applications such as word processing software (e.g., Microsoft Word™ or Corel WordPerfect™) and database software (e.g., spreadsheet software such as Microsoft Excel™, Corel Quattro Pro™, or database programs such as Microsoft Access™ or Paradox™) can be adapted to the present invention by inputting a  
5 character string corresponding to the lipase homologues of the invention (either nucleic acids or proteins, or both). For example, the integrated systems can include the foregoing software having the appropriate character string information, e.g., used in conjunction with a user interface (e.g., a GUI in a standard operating system such as a Windows, Macintosh or  
10 LINUX system) to manipulate strings of characters. As noted, specialized alignment programs such as BLAST can also be incorporated into the systems of the invention for alignment of nucleic acids or proteins (or corresponding character strings).

Integrated systems for analysis in the present invention typically include a digital computer with GA software for aligning sequences, as well as data sets entered into the software system comprising any of the sequences herein. The computer can be, e.g., a  
15 PC (Intel x86 or Pentium chip- compatible DOS™, OS2™ WINDOWS™ WINDOWS NT™, WINDOWS95™, WINDOWS98™ LINUX based machine, a MACINTOSH™, Power PC, or a UNIX based (e.g., SUN™ work station) machine) or other commercially common computer which is known to one of skill. Software for aligning or otherwise manipulating sequences is available, or can easily be constructed by one of skill using a  
20 standard programming language such as Visualbasic, Fortran, Basic, Java, or the like.

Any controller or computer optionally includes a monitor which is often a cathode ray tube ("CRT") display, a flat panel display (e.g., active matrix liquid crystal display, liquid crystal display), or others. Computer circuitry is often placed in a box which includes numerous integrated circuit chips, such as a microprocessor, memory, interface  
25 circuits, and others. The box also optionally includes a hard disk drive, a floppy disk drive, a high capacity removable drive such as a writeable CD-ROM, and other common peripheral elements. Inputting devices such as a keyboard or mouse optionally provide for input from a user and for user selection of sequences to be compared or otherwise manipulated in the relevant computer system.

The computer typically includes appropriate software for receiving user instructions, either in the form of user input into a set parameter fields, e.g., in a GUI, or in the form of preprogrammed instructions, e.g., preprogrammed for a variety of different specific operations. The software then converts these instructions to appropriate language for  
5 instructing the operation of, e.g., fluid direction and transport controllers to carry out the desired operation.

The software can also include output elements for controlling nucleic acid synthesis (e.g., based upon a sequence or an alignment of a sequences herein) or other operations which occur downstream from an alignment or other operation performed using a  
10 character string corresponding to a sequence herein.

In one embodiment, the invention provides an integrated system comprising a computer or computer readable medium comprising a database having one or more sequence records. Each of the sequence records comprises one or more character strings  
15 corresponding to a nucleic acid or polypeptide or protein sequence selected from SEQ ID NO: 1 to SEQ ID NO: 108. The integrated system further comprises a user input interface allowing a user to selectively view the one or more sequence records. In one such integrated system, the computer or computer readable medium comprises an alignment instruction set that aligns the character strings with one or more additional character strings corresponding to a nucleic acid or polypeptide or protein sequence.

20 One such integrated system includes an instruction set that comprises at least one of the following: a local sequence comparison or a local homology comparison determination, a sequence alignment or a homology alignment determination, a sequence identity or similarity search or a search for similarity determination, a sequence identity or similarity determination, a structural similarity search, a structure determination, a nucleic  
25 acid motif determination, an amino acid motif determination, a hypothetical translation, a determination of a restriction map, a sequence recombination and a BLAST determination. In some embodiments, the system further comprises a readable output element that displays an alignment produced by the alignment instruction set. In another embodiment, the computer or computer readable medium further comprises an instruction set that translates at  
30 least one nucleic acid sequence which comprises a sequence selected from SEQ ID NO: 1 to



SEQ ID NO: 54 into an amino acid sequence. The instruction set may select the nucleic acid by applying a codon usage instruction set or an instruction set which determines sequence identity to a test nucleic acid sequence.

Methods of using a computer system to present information pertaining to at least one of a plurality of sequence records stored in a database are also provided. Each of the sequence records comprises at least one character string corresponding to SEQ ID NO: 1 to SEQ ID NO: 108. The method comprises determining at least one character string corresponding to one or more of SEQ ID NO: 1 to SEQ ID NO: 108 or a subsequence thereof; determining which of the at least one character string of the list are selected by a user; and displaying each of the selected character strings, or aligning each of the selected character strings with an additional character string. The method may further comprise displaying an alignment of each of the selected character strings with an additional character string and/or displaying the list.

The current invention provides a database of one or more character strings corresponding to polynucleotide sequences selected from SEQ ID NO: 1 to SEQ ID NO: 54 or a polypeptide sequence selected from SEQ ID NO: 55 to SEQ ID NO: 108. Such database optionally comprises wherein one or more character string is recorded in a computer readable medium (e.g., that resides internal or external to a computer). The invention also provides a method for manipulating a sequence record in a computer system by reading a character string corresponding (optionally selected by a user or wherein the user selects the character string from a database or inputs the character string into the computer system) to a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54 or a polypeptide sequence selected from SEQ ID NO: 55 to SEQ ID NO: 108 (or a subsequence thereof), performing an operation on the character string, and returning a result of the operation (optionally comprising transmitting the selected character string to an output device). The operations performed in such computer system optionally comprise any of the following: a local sequence comparison, a sequence alignment, a sequence identity or similarity search, a structural similarity search, a sequence identity or similarity determination, a structure determination, a nucleic acid motif determination, an amino acid motif determination, a hypothetical translation, a determination of a restriction map, a sequence recombination, or a

BLAST determination. Such method can comprise aligning the selected character string with one or more additional character strings corresponding to a polynucleotide or polypeptide sequence; translating one or more character strings from SEQ ID NO: 1 to SEQ ID NO: 54 into a character string corresponding to an amino acid sequence or translating a character string selected from SEQ ID NO: 55 to SEQ ID NO: 108, into a character string  
5 corresponding to a polynucleotide sequence; determining sequence identity or similarity between the selected character string and one or more additional character strings by evaluating codon usage (optionally determining optimal codon usage); and obtaining the result of the operation on a user output device (e.g., optionally selected from a display  
10 monitor, a printer, and an audio output). The method of the invention for manipulating a sequence record in a computer system also comprises wherein the operation transmits the character string to a device (e.g., an oligonucleotide synthesizer or peptide synthesizer) capable of producing a physical embodiment of the character string (e.g., a physical embodiment comprising a nucleic acid or polypeptide or peptide corresponding to a character  
15 string or a sub-portion thereof)

#### KITS

In an additional aspect, the present invention provides kits embodying the methods, composition, systems and apparatus herein. Kits of the invention optionally comprise one or more of the following: (1) an apparatus, system, system component or  
20 apparatus component as described herein; (2) instructions for practicing the methods described herein, and/or for operating the apparatus or apparatus components herein and/or for using the compositions herein; (3) one or more lipase composition or component; (4) a container for holding components or compositions, and, (5) packaging materials.

In a further aspect, the present invention provides for the use of any apparatus,  
25 apparatus component, composition or kit herein, for the practice of any method or assay herein, and/or for the use of any apparatus or kit to practice any assay or method herein.

## EXAMPLES

### EXAMPLE I: DETECTION OF LIPASE SECRETING BACTERIA

As described above, the nucleic acid and amino acid sequence of SEQ ID NO: 1 through SEQ ID NO: 20 and SEQ ID NO: 55 through SEQ ID NO: 74 were discovered and isolated in a number of *Bacillus* species (both species-typed and un-typed species). In order to choose *Bacillus* cultures that expressed lipase activity, two types of plate assays were performed.

The first type of plate assay comprised a rhodamine B assay (*see, e.g., Kouker, G. et al., Specific and sensitive plate assay for bacterial lipases, Appl Environ Microbiol, (1987) 53:211-213.* The assay entails preparing TGY media plates, onto which various *Bacillus* colonies were patched. The TGY media plates were prepared by mixing 5g tryptone, 5g yeast extract, 5g dextrose, and 1g K<sub>2</sub>HPO<sub>4</sub> per liter of media. The media was autoclaved and cooled to approximately 60°C before 30 milliliters of filtered sterilized soybean oil and 2 milliliters of filtered sterilized rhodamine B solution (0.1%) was vigorously mixed in. The media was then plated into petri dishes.

If the *Bacillus* colonies that were patched onto the TGY plates secreted active lipase enzymes, such enzymes would act upon the soybean oil in the plates, thus releasing free fatty acids. The free fatty acids would then react with the rhodamine B to create a visible fluorescent orange compound. Thus, *Bacillus* colonies that expressed active lipase could be visually detected (after 24-48 hours) by the fluorescent orange halo around the colonies.

The second type of plate based assay used to detect the presence of lipase activity was used to check for lipase activity of *E. coli* bacterial colonies. *E. coli* cultures were transformed with expression vectors containing either the newly discovered *Bacillus* lipase variants (*e.g., as detected above*) or with newly created (*i.e., recombined*) lipase homologue variants. The transformed *E. coli* colonies were grown on plates containing LB media supplemented with tributyrin at a final concentration of 1%. Colonies expressing an active lipase, secreted such lipase into the surrounding media (which was hazy due to the tributyrin), thus, degrading the tributyrin and producing a clear media ring around the lipase active colonies.

EXAMPLE II: SCREENING LIPASE HOMOLOGUES FOR ENANTIOSELECTIVITY

## A. Substrate Synthesis

All materials were purchased from Sigma or Aldrich unless noted. Neryl butyrate was prepared by from nerol and butyryl chloride in methylene chloride/pyridine.

5 Geranyl deuterobutyrate was prepared from geraniol and deuterobutyric acid (Isotec) using DCC coupling in methylene chloride. Both compounds were purified by flash chromatography (ether/hexanes) and gave satisfactory analysis by mass spectrometry and NMR.

## B. Library Pre-Selection and Enzyme Preparation

10 Transformants were robotically picked to 386-well microtiter plates containing 70  $\mu$ L growth medium (2xYT, 0.5% glucose to suppress induction, 30  $\mu$ g/ml chloramphenicol) and grown 12-20 hours at 37°C, 300-rpm shaking speed in a Kuhner incubator. The cultures were then gridded via a Q-bot robot (Genetix, UK) to inducing agar (2xYT, 1.5% agar, 1mM IPTG, 30  $\mu$ g/ml chloramphenicol) in 22 cm x 22 cm bioassay trays using 0.25 mm pins, and incubated at 30°C for 16-20 hours. The colonies were then overlaid with substrate (1% neryl butyrate or geranyl butyrate) in 150 mL of 1.5% agar containing 2 mM Hepes, pH 7.4, and 1% Triton X-100 that had been heated to 45°C. The reaction was allowed to proceed at room temperature for 5 to 20 hours, until clearing zones around active colonies were visible. The trays were imaged against a black background with an Alpha  
20 Innotech Fluorchem imaging system, and the images were analyzed using Phoretix Array image analysis software. Active clones were identified based upon the intensity of the corresponding clearing zone, and transferred (5  $\mu$ L) from the master 384-well plates to rows 1-7 of 96 well microtiter plates containing 200  $\mu$ L growth medium. The final row of the 96-well plate was spiked with 5  $\mu$ L cultures transformed with a plasmid that did not contain an  
25 active lipase as a negative background control. The cultures were grown overnight at 37°C at 200-230 rpm shaking speed in a Kuhner incubator. The following day, 10  $\mu$ L of each culture was dispensed into 200  $\mu$ L inducing media (2xYT, 1 mM IPTG, 30  $\mu$ g/ml chloramphenicol) in a second 96-well plate. The cultures were induced for 16-20 hours at 30°C, 200 rpm in a Kuhner incubator. The cells were then pelleted by centrifugation and the lipase-containing  
30 supernatant assayed as described below.

C. Reactions, Mass Spectrometrical Analysis, and Results

Ten  $\mu\text{L}$  of cell supernatant was added to 90  $\mu\text{L}$  reaction mix that contained 2.78 mM neryl butyrate, 2.78 mM geraniol deuterobutyrate, and 1 mM morpholine acetate, pH 7.4, in a 96-well plate. The plates were sealed with plastic tape and shaken on a  
5 MicroMix (Diagnostics Products Corporation) set to mix at amplitude 4, form 20. After 8 hours, 10  $\mu\text{L}$  of this reaction mix was added to 90  $\mu\text{L}$  40:50  $\text{H}_2\text{O}$ :MeOH. The final row of the plate was spiked with known concentrations of butyrate and deuterobutyrate (0 - 50  $\mu\text{M}$ ) to provide calibration curves. The plates were sealed (MicroLiter Analytical polypropylene & aluminum foil film) and analyzed by LC/MS for butyrate and deuterobutyrate  
10 concentrations. Clones showing desired specificity were then re-confirmed by GC/MS.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the  
15 true scope of the invention. For example, all the techniques, methods, compositions, apparatus and systems described above may be used in various combinations. *All publications, patents, patent applications, or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, or other document were individually*  
20 *indicated to be incorporated by reference for all purposes.*

## SEQUENCE LISTINGS

SEQ ID NO	CLONE NAME	SEQUENCE
SEQ ID NO: 1	405 (pumilus)	ATGAAATTTGTAAAAAGAAGGATCATTGCACT TGTAACAATTTTGGTGCTGTCAGTCACATCGC TGTTTGGGATGCAGCCGTCAGCAAAGCCGCT GAACACAATCCAGTTGTTATGGTTCACGGTAT CGGAGGAGCTTCATACAATTTGCGGGAATTA AGAGCTATCTCGTATCTCAGGGCTGGTCACGG GGCAAGCTGTATGCGGTTGATTTTGGGACAA GACAGGGACGAATTATAACAATGGCCCGGTAT TATCACGATTTGTGCAAAAGGTTTATAGACGAA ACGGGTGCMAAAAAGTGGATATTGTCGCTCA CAGTATGGGTGGCGCGAACACACCTTACTACA TAAAAATCTGGACGGCGGAAATAAAATTGAA AACGTCGTAACGCTTGGCGGCGGAACCGTTC GACGACAAGCAAGGCGCTTCCGGGAACAGATC CAAATCAAAGATTTTATAACACATCCATTTAC AGCAGTGCCGATATGATTGTCATGAATTACTT ATCAAATTAGACGGTGCTAAAAACGCTCAA TTCATGGCGTTGGGCACATTGGTTTATTGATG AACAGCCAAGTCAACAGCCTGATTAAGAAGG ACTGAACGGCGGGGGCCAAAATACGAATTA
SEQ ID NO: 2	406 (subtilis)	ATGAAATTTGTAAAAAGAAGGATCATTGCACT TGTAACAATTTTGTATGCTGTCTGTTACATCGC TGTTTGGGTTGCAGCCGTCAGCAAAGCCGCT GAACACAATCCAGTCGTTATGGTTCACGGTAT TGGAGGGGCATCATTCAATTTGCGGGAATTA AGAGCTATCTCGTATCTCAGGGCTGGTCGCGG GACAAGCTGTATGCAGTTGATTTTGGGACAA GACAGGCACAAATTATAACAATGGACCGGTAT TACCACGATTTGTGCAAAAGGTTTATAGATGAA ACGGGTGCMAAAAAGTGGATATTGTCGCTCA CAGCATGGGGGGCGCGAACACACTTACTACA TAAAAATCTGGACGGCGGAAATAAAGTTGCA AACGTCGTGACGCTTGGCGGCGGAACCGTTT GACGACAGGCAAGGCGCTTCCGGGAACAGATC CAAATCAAAGATTTTATAACACATCCATTTAC AGCAGTGCCGATATGATTGTCATAAATTACTT ATCAAGATTAGATGGTGCTAGAAACGTTCAA TCCATGGCGTTGGACACATCGGCCTTCTGTAC AGCAGCCAAGTCAACAGCCTGATTAAGAAGG GCTGAACGGCGGGGGACTCAATACAAATTAG
SEQ ID NO: 3	402 (megaterium)	ATGAAATTTGTAAAAAGAAGGATCATTGCACT TGTAACAATTTTGGTGCTGTCAGTCACATCGC

		<p>TGTTTGGGATGCAGCCGTCAGCAAAGCCGCT  GACACAATCCAGTTGTTATGGTTCACCTGGTAT  CGGAGGAGCTTCATACAATTTTGC GGGAATTA  AGAGCTATCTCGTATCTCAGGGCTGGTCACGG  GGCAAGCTGTATGCGGTTGATTTTTGGGACAA  GACAGGGACGAATTATAACAATGGCCCGGTAT  TATCACGATTTGTGCAAAAAGGTTTTAGACGAA  ACGGGTGCGAAAAAAGTGGATATTGTCGCTCA  CAGCATGGGTGGCGCGAACACACTTTACTACA  TAAAAAATCTGGACGGCGGAAATAAAATTGAA  AACGTCGTAACGCTTGGCGGCGGAACCGTTT  GACGACAAGCAAGGCGCTTCCGGGAACAGATC  CAAATCAAAAAGATTTTATACACATCCATTTAC  AGCAGTGCCGATATGATTGTCATGAATTACTT  ATCAAAATTAGACGGTGCTAAAAACGTTCAAAA  TTCATGGCGTTGGGCACATTTGGTTTATTGATG  AACAGCCAAGTCAACAGCCTGATTAAGAAGG  ACTGAACGGCGGGGGCCACAATACAAATTAA</p>
<p>SEQ ID NO: 4</p>	<p>400 (lentus)</p>	<p>ATGAAATTTGTA AAAAGAAGGATCATTGCACT  TGTAACAATTTTGGTGCTGTCAGTCACATCGC  TGTTTGGGATGCAGCCGTCAGCAAAGCCGCT  GAACACAATCCAGTTGTTATGGTTCACGGTAT  CGGAGGAGCTTCATACAATTTTGC GGGAATTA  AGAGCTATCTCGTATCTCAGGGCTGGTCACGG  GGCAAGCTGTATGCGGTTGATTTTTGGGACAA  GACAGGGACGAATTATAACAATGGCCCGGTAT  TATCACGATTTGTGCAAAAAGGTTTTAGACGAA  ACGGGTGCGAAAAAAGTGGATATTGTCGCTCA  CAGCATGGGTGGCGCGAACACACTTTACTACA  TAAAAAATCTGGACGGCGGAAATAAAATTGAA  AACGTCGTAACGCTTGGCGGCGGAACCGTTT  GACGACAAGCAAGGCGCTTCCGGGAACAGATC  CAAATCAAAAAGATTTTATACACATCCATTTAC  AGCAGTGCCGATATGATTGTCATGAATTACTT  ATCAAAATTAGACGGTGCTAAAAACGTTCAAAA  TTCATGGCGTTGGGCACATTTGGTTTATTGATG  AACAGCCAAGTCAACAGCCTGATTAAGAAGG  ACTGAACGGCGGAGGACTAAATACAAATTAA</p>
<p>SEQ ID NO: 5</p>	<p>396 (circulans)</p>	<p>ATGAAATTTATA AAAAGAAGGATCATTGCACT  TGTAACAATTTTGGTGCTGTCAGTCACATCGC  TGTTTGGGATGCAGCCGTCAGCAAAGCCGCT  GAACACAATCCAGTTGTTATGGTTCACGGTAT  CGGAGGAGCTTCATACAATTTTGC GGGAATTA  AGAGCTATCTCGTATCTCAGGGCTGGTCACGG  GGCAAGCTGTATGCGGTTGATTTTTGGGACAA  GACAGGGACGAATTATAACAATGGCCCGGTAT</p>

		<p>TATCACGATTTGTGCAAAAAGGTTTTAGACGAA                  ACGGGTGCAGAAAAAGTGGATATTGTCGCTCA                  CAGCATGGGTGGCGCGAACACACTTTACTACA                  TAAAAAATCTGGACGGCGGAAATAAAATTGAA                  AACGTCGTAACGCTTGGCGGCGCGAACCGTTT                  GACGACAAGCAAGGCGCTTCCGGGAACAGATC                  CAAATCAAAGATTTTTATACACATCCATTTAC                  AGCAGTGCCGATATGATTGTCATGAATTACTT                  ATCAAATTAGACGGTGCTAAAAACGTTCAAA                  TTCATGGCGTTGGGCACATTGGTTTTATTGATG                  AACAGCCAAGTCAACAGCCTGATTAAAGAAGG                  ACTGAACGGCGGGGGCCTCAATACAAATTAA</p>
<p>SEQ ID NO: 6</p>	<p>392 (azotoformans)</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT                  TGTAACAATTTTGGTGCTGTCAGTCACATCGC                  TGT'TTGGGATGCAGCCGTCAGCAAAGCCGCT                  GAACACAATCCAGTTGTTATGGTTCACGGTAT                  CGGAGGAGCTTCATACAATTTTGGCGGAATTA                  AGAGCTATCTCGTATCTCAGGGCTGGTCACGG                  GGCGAGCTGTATGCGGTTGATTTTTGGGACAA                  GACAGGGACGAATTATAACAATGGCCCGGTAT                  TATCACGATTTGTGCAAAAAGGTTTTAGACGAA                  ACGGGTGCAGAAAAAGTGGATATTGTCGCTCA                  CAGCATGGGTGGCGCGAACACACTTTACTACA                  TAAAAAATCTGGACGGCGGAAATAAAATTGAA                  AACGTCGTAACGCTTGGCGGCGCGAACCGTTT                  GACGACAAGCAAGGCGCTTCCGGGAACAGATC                  CAAATCAAAGATTTTTATACACATCCATTTAC                  AGCAGTGCCAATATGATTGTCATGAATTACTT                  ATCAAATTAGACGGTGCTAAAAACGTACAAA                  TTCATGGCGTTGGGCACATTGGTTTTATTGATG                  AACAGCCAAGTCAACAGCCTGATTAAAGAAGG                  ACTGAACGGCGGGGGCCTAGATACAAATTAA</p>
<p>SEQ ID NO: 7</p>	<p>398 (firmus)</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT                  TGTAACAATTTTGGTGCTGTCAGTCACATCGC                  TGT'TTGGGATGCAGCCGTCAGCAAAGCCGCT                  GAACACAATCCAGTTGTTATGGTTCACGGTAT                  CGGAGGAGCTTCATACAATTTTGGCGGAATTA                  AGAGCTATCTCGTATCTCAGGGCTGGTCACGG                  GGCAAGCTGTATGCGGTTGATTTTTGGGACAA                  GACAGGGACGAATTATAACAATGGCCCGGTAT                  TATCACGATTTGTGCAAAAAGGTTTTAGACGAA                  ACGGGTGCAGAAAAAGTGGATATTGTCGCTCA                  CAGCATGGGTGGCGCGAACACACTTTACTACA                  TAAAAAATCTGGACGGCGGAAATAAAATTGAA                  AACGTCGTAACGCTTGGCGGCGCGAACCGTTT                  GACGACAAGCAAGGCGCTTCCGGGAACAGATC</p>



		<p>CAAATCAAAGATTATACACATCCATTTAC                  AGCAGTGCCGATATGATTGTCATGAATTACTT                  ATCAAATTAGACGGTGCTAAAAACGCTCAA                  TTCATGGCGTTGGGCACATGGTTTATTGATG                  AACAGCCAAGTCAACAGCCTGATTAAGAAGG                  ACTGAACGGCGGAGGCCACAATACAAATTA</p>
<p>SEQ ID NO: 8</p>	<p>393 (badius)</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT                  TGTAACAATTTTGGTGCTGTCAGTCACATCGC                  TGTTTGCGATGCAGCCGTCAGCAAAGCCGCT                  GAACACAATCCAGTTGTTATGGTTCACGGTAT                  CGGAGGAGCTTCATACAATTTTGCGGGAATTA                  AGAGCTATCTCGTATCTCAGGGCTGGTCACGG                  GGCAAGCTGTATGCGGTTGATTTTTGGGACAA                  GACAGGGACGAATTATAACAATGGCCCGGTAT                  TATCACGATTTGTGCAAAAGGTTTTAGACGAA                  ACGGGTGCGAAAAAGTGGATATTGTGCGCTCA                  CAGCATGGGTGGCGCGAACACACTTTACTACA                  TAAAAATCTGGACGGCGGAAATAAATTTGAA                  AACGTCGTAACGCTTGGCGGCGCAACCGTTT                  GACGACAAGCAAGGCGCTTCCGGGAACAGATC                  CAAATCAAAGATTATACACATCCATTTAC                  AGCAGTGCCGATATGATTGTCATGAATTACTT                  ATCAAATTAGACGGTGCTAAAAACGTTCAA                  TTCATGGCGTTGGGCACATGGTTTATTGATG                  AACAGCCAAGTCAACAGCCTGATTAAGAAGG                  ACTGAACGGCGGAGGCCACAATACAAATTA</p>
<p>SEQ ID NO: 9</p>	<p>Dc5h</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT                  TGTAACAATTTTGTATGCTGCTGTTACATCGC                  TGTTTGCGTTGCAACCGTCAGCAAAGCCGCT                  GAACACAATCCAGTCGTTATGGTTCACGGTAT                  TGGAGGGGCATCATTCAATTTTGCGGGAATTA                  AGAGCTATCTCGTATCTCAGGGCTGGTCGCGG                  GACAAGCTGTATGCAGTTGATTTCAAGGACAA                  GACAGGCACAAATTATAACAATGGCCCGGTAT                  TATCACGATTTGTGCAAAAGGTTTTAGATGAA                  ACGGGTGCGAAAAAGTGGATATTGTGCGCTCA                  CAGCATGGGGGGCGGAACACACTTTACTACA                  TAAAAATCTGGACGGCGGAAATAAAGTTGAA                  AACGTCGTGACGCTTGGCGGCGCAACCGTTT                  GACGACAGGCAAGGCGCTTCCGGGAACAGATC                  CAAATCAAAGATTATACACATCCATTTAC                  AGCAGTGCCGATATGATTGTCATGAATTATTT                  ATCAAGATTAGATGGTGCGAGAAACGTTCAA                  TCCATGGCGTTGGACACATCGGCCTTCTGTAC                  AGCAGCCAAGTCAACAGCCTGATTAAGAAGG                  GCTGAACGGCGGGGGCTCAATACAAATTA</p>

<p>SEQ ID NO: 10</p>	<p>Dc5f</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT  TGTAACAATTTTGATGCTGTCTGTTACATCGC  TGTTTGCGTTGCAACCGTCAGCAAAAGCCGCT  GAACACAATCCAGTCGTTATGGTTCACGGTAT  TGGAGGGGCATCATTCAATTTTGC GGGAATTA  AGAGCTACCTCGTATCTCAGGGCTGGTTCGCGG  GACAAGCTGTATGCAGTTGATTTCTAAGACAA  AACAGGGAATAACCGCAACAATGGTCCGCGTC  TATCGAGATTCGTCAAAGATGTGTTAGACAAA  ACGGGTGCCAAAAAAGTAGATATTGTGGCTCA  TAGTATGGGTGGAGCGAACACGCTATACTATA  TCAAGAATCTAGATGGCGGCGATAAAAATTGAG  AACGTTGTCACAATTGGTGGAGCAAACGGACT  CGTTTCAAGCAGAGCATTACCAGGCACAGATC  CAAATCAAAAAATTCTTTACACATCCGTCTAT  AGCTCAGCAGATCTTATTGTCGTCAACAGCCT  CTCTCGTTTAATTGGCGCAAGAAACATCCTGA  TCCATGGCGTTGGTCATATCGGTCTATTAACC  TCAAGCCAAGTGAAAGGGTATATTAAGAAGG  ACTGAACGGCGGAGGCCTCAATACAAATTAA</p>
<p>SEQ ID NO: 11</p>	<p>Dc5c1</p>	<p>ATGAAAGTGATTTTTGTAAAGAAAAGGAGTTT  GCAAATTC TTGTTGCCCTTGCCTTAGTGCTAG  GTTCAATAGCCTTCATCCAGCCGAAAGAAGCC  AAAGCGGCTGAGCATAATCCGGTTGTAATGGT  GCATGGCATGGGTGGTGCCTTTATAACTTTG  CTTCGATCAAACGATACTTAGTATCACAGGGA  TGGGATCAAACCAACTTTTGTCAATCGATTT  CATAGACAAAACAGGCAATAACCTAAACAATG  GCCCCGAGGCTCTCGAGATTCGTGAAAGACGTA  CTAGCCAAAACGGGCGCCAAAAAAGTAGATAT  TGTGGCTCATAGTATGGGCGGTGCGAACACGT  TATACTATATTA AAAACCTAGACGGTGGAGAT  AAAATTGAAAACGTCGTCACATTAGGTGGAGC  AAACGGACTCGTATCACTCAGAGCATTACCAG  GCACCGATCCAAATCAAAAAATTCTTTACACA  TCTGTCTATAGCTCAGCCGATCTCATTGTCGT  CAACAGCCTTTCGCGTTTAATTGGCGCAAGAA  ACGTCCGTGATCCACGGCGTTGGACATATCGGT  CTATTAACCTCAAGCCAAGTCAAAGGCTATGT  GAAAGAAGGATTGAATGGCGGGGACAGAATA  CAAATTAA</p>
<p>SEQ ID NO: 12</p>	<p>Dc5a2</p>	<p>ATGAAAGTGATTTTTGTAAAGAAAAGGAGTTT  GCAAATTC TTGTTGTGCTTGCAATTGGTGATGG  GTTCAATGGCCTTCATCCAGCCAAAAGAGATC  AGAGCGGCTGAGCATAATCCGGTTGTGATGGT  ACATGGCATGGGCGGTGCGTCTTATAACTTTG</p>

		<p>CTTCGATTAAAAGTTACTTGGTATCACAAGGA  TGGGATCGAAACCAATTATTTGCTATCGATTT  CATAGACAAAACAGGTAATAACCGCAACAATG  GTCCGCGTCTATCCAGATTCGTCAAAGATGTG  CTAGCCAAAACAGGTGCCAAAAAAGTTGATAT  TGTGGCTCATAGTATGGGCGGAGCGAACACGT  TATACTATATTAAGAATCTAGACGGCGGCGAT  AAAATAGAAAACGTTGTTACACTTGGTGGAGC  GAACGGACTCGTTTCACTCAGAGCATTACCAG  GCACCGATCCAAATCAAAAAATCCTTTACACA  TCCGTCTACAGCTCAGCCGATCTTATCGTCGT  CAACAGCCTCTCGCGTTTAATTTGGCGCAAGAA  ACGTCCATTCACGGCGTTGGTCACATCGGT  CTATTAGCTTCAAGCCAAGTCAAAGGCTATAT  CAAAGAAGGACTGAATGGCGGAGGCCAAAATA  CAAATTAA</p>
<p>SEQ ID NO: 13</p>	<p>Dc512</p>	<p>ATGAAAGTGATTTTTGTTAAGAAAAGGAGTTT  GCAAATTCCTCATTTGCGCTTGCATTTGGTGATTG  GTTCAATGGCGTTTATCCAGCCGAAAGAGGCG  AAGGCGGCTGAGCATAATCCGGTTGTGATGGT  GCATGGCATTTGGCGGTGCCTCTTATAACTTTT  TTTCTATTTAAAAGTTATTTGGCCACACAAGGC  TGGGATCGAAACCAATTATATGCTATTGATTT  CATAGACAAAACAGGAAATAACCGCAACAATG  GTCCGCGTCTATCGAGATTCGTCAAAGATGTG  TTAGACAAAACGGGTGCCAAAAAAGTAGATAT  TGTGGCTCATAGTATGGGTGGAGCGAACACGC  TATACTATATCAAGAATCTAGATGGCGGCGAT  AAAATTGAGAACGTTGTCACAATTGGTGGAGC  AAACGGACTCGTTTCAAGCAGAGCATTACCAG  GCACAGATCCAAATCAAAAAATCCTTTACACA  TCCGTCTATAGCTCAGCAGATCTTATTGTCTGT  CAACAGCCTCTCTCAGTTTAATTTGGCGCAAGA  AACATCCTGATCCAGGCGTTGGTCATATCGGT  CTATTAACCTCAAGCCAAGTCAAAGGCTATAT  TAAAGAAGGACTGAACGGCGGAGGCCCTCAATA  CAAATTAA</p>
<p>SEQ ID NO: 14</p>	<p>Sga</p>	<p>ATGAAATTTGTAAAAGAAGGATCATTGCACT  TGTAACAATTTTGATGCTGTCTGTTACATCGC  TGTTTGCCTTGCAACCGTCAGCAAAGCCGCT  GAACACAATCCAGTCGTTATGGTTCACGGTAT  TGGAGGGGCATCATTCAATTTTGCGGGAATTA  AGAGCTATCTCGTATCTCAAGGCTGGTTCGCGG  GACAAGCTGTATGCAGTTGATTTCAAGGACAA  GACAGGCAATAACTTAAACAACGGTCCAGTAT  TATCGCGTTTCGTGAAAAGGTATTAGATGAA</p>

		<p>ACCGGTGCGAAAAAAGTGGATATTGTCGCTCA                  CAGCATGGGCGGCGCTAACACGCTTTACTACA                  TAAAAAATTTGGATGGCGGTAATAAAATTGAA                  AACGTCGTAACACTTGGCGGCGGAATCGTCT                  TGTGACAGGCAAGGCGCTTCCGGGTACTGATC                  CCAACCAAAGATCTTGTACACATCCGTTTAC                  AGTAGTGCTGATATGATTGTTATGAATTACTT                  AACAAAATTAGACGGGGCTAAAAATGTTCAA                  TTCATGGTGTCCGACATATCGGCCTTCTGTAC                  AGCAGCCAAGTCAACAGCCTGATTAAGAAGG                  GCTTAACGGCGGAGGCCTCAATACAAATTAA</p>
<p>SEQ ID NO: 15</p>	<p>Sgc</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT                  TGTAACAATTTTGATGCTGCTGTTACATCGC                  TGTTTGCGTTGCAACCGTCAGCAAAGCCGCT                  GAACACAATCCAGTCGTTATGGTTCACGGTAT                  TGGAGGGGCATCATTCAATTTTGCGGGAATTA                  AGAGCTATCTCGTATCTCAGGGCTGGTCCGGG                  GACAAGCTGTATGCAGTTGATTTCTGGGATAA                  GACAGGCAATAACTTAAACAACGGTCCAGTAT                  TATCGCGTTTTGTGAAAAAGGTATTAGATGAA                  ACCGGTGCGAAAAAAGTGGATATTGTCGCTCA                  CAGCATGGGCGGCGCTAACACGCTTTACTACA                  TAAAAAATTTGGATGGCGGTAATAAAATTGAA                  AACGTCGTAACACTTGGCGGCGGAATCGTCT                  TGTGACAGGCAAGGCGCTTCCGGGTACTGATC                  CCAACCAAAGATATTGTACACATCCGTTTAC                  AGTAGTGCTGATATGATTGTTATGAATTACTT                  ATCAAATTTAGACGGGGCTAAAAATGTTCAA                  TTCATGGTGTCCGACATATCGGCCTTCTGTAC                  AGCAGCCAAGTCAATAGCCTGATTAAGAAGG                  GCTTAACGGCGGAGGACTCAATACGAATTAA</p>
<p>SEQ ID NO: 16</p>	<p>Sgd</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT                  TGTAACAATTTTGATGCTGCTGTTACATCGC                  TGTTTGCGTTGCAACCGTCAGCAAAGCCGCT                  GAACACAATCCAGTCGTTATGGTTCACGGTAT                  TGGAGGGGCATCATTCAATTTTGCGGGAATTA                  AGAGCTATCTCGTATCTCAGGGCTGGTCCGGG                  GACAAGCTGTATGCAGTTGATTTTAGTGACAA                  AACAGGCAATAACTTAAACAACGGTCCAGTAT                  TATCGCGTTTTGTGAAAAAGGTATTAGATGAA                  ACCGGTGCGAAAAAAGTGGATATTGTCGCTCA                  CAGCATGGGCGGCGCTAACACGCTTTACTACA                  TAAAAAATTTGGATGGCGGTAATAAAATTGAA                  AACGTCGTAACACTTGGCGGCGGAATCGTCT                  TGTAACAGGCAAGGCGCTTCCGGGTACTGATC                  CCAACCAAAGATCTTGTACACATCCGTTTAC</p>

		<p>AGTAGTGCTGATATGATTGTTATGAATTACTT                  ATCAAATTTAGACGGGGCTAAAAATGTTCAA                  TTCATGGTGTCTGGACATATCGGCCTTCTGTAC                  AGCAGCCAAGTCAACAGCCTGATTAAGAAGG                  GCTTAACGGCGGGGGCCTGAATACGAATTAA</p>
<p>SEQ ID NO: 17</p>	<p>Sgf</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT                  TGTAACAATTTTGATGCTGTCTGTTACATCGC                  TGTTTGGCGTTGCAACCGTCAGCAAAGCCGCT                  GAACACAATCCAGTCGTTATGGTTCACGGTAT                  TGGAGGGGCATCATTC AATTTGCGGGAATTA                  AGAGCTATCTCGTATCTCAGGGCTGGTCGCGG                  GACAAGCTGTATGCAGTTGATTTCAAAGACAA                  GACAGGGAATAACCGCAACAATGGTCCGCGTC                  TATCGAGATTCGTCAAAGATGTGTTAGACAAA                  ACAGGAGCCAAAAAAGTAGATATTGTGGCTCA                  TAGTATGGGCGGAGCGAACACATTATACTATA                  TTAAGAATCTAGATGGTGGCGATAAAATTGAG                  AACGTTGTCACAATTTGGTGGAGCAAACGGACT                  CGTTTCAAGCAGAGCATTACCAGGCACAGATC                  CAAATCAAAAAATTC TTTACACATCCGTCTAT                  AGCTCAGCAGATCTTATTGTCGTCAACAGTCT                  CTCTCGTTTAATTGGCGCAAGAAACGTCCAAA                  TCCATGGCGTTGGACATATCGGTCTATTAACC                  TCAAGCCAAGTCAAAGGATATATTAAGAAGG                  ACTGAACGGCGGGGGCCTCAATACAAATTAA</p>
<p>SEQ ID NO: 18</p>	<p>Sgh</p>	<p>ATGAAATTTGTAAAAAGAAGGATCCTTGC ACT                  TGTAACAATTTTGATGCTGTCTGTTACATCGC                  TGTTTGGCGTTGCAACCGTCAGCAAAGCCGCT                  GAACACAATCCAGTCGTTATGGTTCACGGTAT                  TGGAGGGGCATCATTC AATTTGCGGGAATTA                  AGAGCTATCTCGTATCTCAGGGCTGGTCGCGG                  GACAAGCTGTATGCAGTTGATTTCA TTGACAA                  GACAGGAAATAACCGCAACAATGGTCCGCGTC                  TATCGAGATTCGTCAAAGATGTGTTAGACAAA                  ACAGGAGCCAAAAAAGTAGATATTGTGGCTCA                  TAGTATGGGCGGAGCGAACACATTATACTATA                  TTAAGAATCTAGATGGTGGCGATAAAATTGAG                  AACGTTGTCACAATTTGGTGGAGCAAACGGACT                  CGTTTCAAGCAGAGCATTACCAGGCACAGATC                  CAAATCAAAAAATTC TTTACACATCCGTCTAT                  AGCTCAGCAGATCTTATTGTCGTCAACAGTCT                  CTCTCGTTTAATTGGCGCAAGAAACGTCCAAA                  TCCATGGCGTTGGACATATCGGTCTATTAACC                  TCAAGCCTAGTCAAAGGATATATTAAGAAGG                  ACTGAACGGCGGAGGCCAAAAATACAAATTAA</p>

<p>SEQ ID NO: 19</p>	<p>Mt2b1</p>	<p>ATGAAAGTGATTTTTGTTAAGAAAAGGAGTTT  GCAAATTCCTTGTGTCCTTGCCTTAGTGATAG  GTTCAATGGCCTTCATCCAGCCAAAAGAAATC  AAAGCAGCTGAGCACAATCCGGTTGTGATGGT  ACATGGTATTGGAGGAGCGTCTTATAACTTTG  CTTCGATTA AAAAGTTATTTGGTTAACCAAGGC  TGGGATCGAAAACCAATTATTTGCTATCGATTT  CATAGACAAAACAGGGAATAACCGCAACAATG  GTCCTCGTTTATCTAGATTCGTCAAAGATGTG  CTAGACAAAACGGGTGCCAAAAAAGTAGATAT  TGTGGCGCATAGTATGGGCGGGCGAACACGC  TATACTATATTAAGAATCTAGATGGCGCGCAT  AAAATTGAAAACGTCGTCACCATTTGGTGGAGC  AAACGGACTCGTTTCACTCAGAGCATTACCAG  GAACAGATCCAAATCAAAAAATCTCTATACA  TCTGTCTATAGCTCAGCCGATTTGATTGTGCT  CAACAGCCTTTCGCGTTTAACTGGCGCAAGAA  ATGTCCTGATCCACGGCGTTGGCCATATCGGT  CTATTAACCTCAAGCCAAGTGAAAGGGTATAT  TAAAGAAGGACTGAACGGCGGGGGCCTAAATA  CAAATTA</p>
<p>SEQ ID NO: 20</p>	<p>H2a</p>	<p>ATGAAATTTGTAAAAAGAAGGATCATTGCACT  TGTAACAATTTTGATGCTGTCTGTTACATCGC  TGTTTGCGTTGCAACCGTCAGCAAAGCCGCT  GAACACAATCCAGTCGTTATGGTTCACGGTAT  TGGAGGGGCATCATTCAATTTTGCGGGAATTA  AGAGCTATCTCGTATCTCAGGGCTGGTTCGCGG  GACAAGCTGTATGCAGTTGATTTACAGGGACAA  GACAGGAAATAACCGCAACAATGGTCCGCGTC  TATCTAAATTCGTCAAAGATGTGTTAGACAAA  ACGGGTGCCAAAAAAGTAGATATTGTGGCTCA  TAGTATGGGCGGGCGAACACGCTATACTATA  TTAAGAATCTAGATGGCGGGGATAAAAATTGAG  AACGTTGTCACAATTTGGCGGAGCAAACGGACT  CGTTTCAAGCAGAGCATTACCAGGCACAGATC  CAAATCAAAAAATCTTTACACATCCGTCTAC  AAGCTCAGCCGATCTCATTGTCTCAACAGTC  TCTCTCGTTTAAATGGCTGCAAGAAACAGTCC  AAATCCATGGCGTTGGACATATCGGTCTATTA  ACCTCAAGCCAAGTCAAAGGATATATTAAAGA  AGGACTGAACGGCGGGGGACTAAATACAAATT  AA</p>
<p>SEQ ID NO: 21</p>	<p>1f15(G2)</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA  TTGGAGGGGCATCATTCAATTTTGCGGGAATTT  AAGAGCTATCTCGTATCTCAGGGCTGGTTCGCG  GGGCAAGCTGTATGCGGTTGATTTTTGGGACA</p>

		<p>AGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCGCGTTTTGTGAAAAAGGTATTAGATGA                  AACGGGTGCGAAAAAAGTGGATATTGTGCGCTC                  ACAGCATGGGCGGCGCTAACACGCTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAAGTTGA                  AAACGTCGTAACGCTTGGCGGCACGAACCGTT                  CGACGACAAGCAAGGCGCTTCCGGGAACAGAT                  CCAAATCAAAAGATTTTATACACATCCATTTA                  CAGCAGTGCCGATATGATTGTCATGAATTACT                  TATCAAAATTAGACGGTGCTAAAAATGTTCAA                  ATTCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAAGAAG                  GACTGAACGGCGGGGGACTCAATACGAATTGA</p>
<p>SEQ ID NO: 22</p>	<p>3C12</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TTGGAGGGGCATCATTCAATTTTGCGGGAATT                  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG                  GGGCAAGCTGTATGCGGTTGATTTTTGGGACA                  AGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCTAGATTCGTCAAAGATGTGCTAGACAA                  AACGGGTGCGAAAAAAGTGGATATTGTGCGCTC                  ACAGCATGGGGGGCGGAACACACTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAATTTGA                  AAACGTCGTAACGCTTGGCGGCAGCAACCGTT                  CGACGACAAGCAAGGCGCTTCCGGGAACAGAT                  CCAAATCAAAAGATTTTATACACATCCATTTA                  CAGCAGTGCCGATATGATTGTCATGAATTACT                  TATCAAAATTAGACGGGGCTAAAAATGTTCAA                  ATTCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAAGAAG                  GACTGAACGGCGGGGGACTCAATACGAATTGA</p>
<p>SEQ ID NO: 23</p>	<p>3N19(G2)</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TTGGAGGGGCATCATTCAATTTTGCGGGAATT                  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG                  GGGCAAGCTGTATGCGGTTGATTTTTGGGACA                  GGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCACGATTTGTGAAAAAGGTATTAGATGA                  AACCGGTGCGAAAAAAGTGGACATTGTGCGCTC                  ACAGCATGGGTGGCGCGAACAACACTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAATTTGA                  AAACGTCGTAACGCTTGGCGGCAGCAACCGTT                  TGACGACAAGCAAGGCGCTTCCGGGAACAGAT                  CCAAATCAAAAGATTTTATACACATCCATTTA                  CGGCAGTGCCGATATGATTGTCATGAATTACT                  TATCAAAATTAGACGGTGCTAAAAACGTTCAA                  ATCCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAAGAAG</p>

<p>SEQ ID NO: 24</p>	<p>G2.2</p>	<p>GACTGAACGGCGGGGGACTGAATACAAATTGA  TGAACACAATCCAGTTGTTATGGTTCACGGTA  TCGGAGGGGCATCATTCAATTTTGCGGGAATT  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG  GGGCAAGCTGTATGCGGTTGATTTTGGGACA  AGACAGGGACGAATTATAACAATGGCCCGGTA  TTATCACGATTTGTGCAAAAGGTTTGTAGACGA  AACGGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGGGGCGGAACACACTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAATTGA  AAACGTCGTAACGCTTGGCGGCGGAACCGTT  CGACGACAAGCAAGGCGCTTCCGGGAACAGAT  CCAAATCAAAGATTTTATACACATCCATTTA  CGGCAGTGCCGATATGATTGTCATGAATTACT  TATCAAAATTAGACGGTGCTAAAAACGTACAA  ATTCATGGCGTTGGGCACATTGGTTTATTGAT  GAACAGCCAAGTCAACAGCCTGATTAAGAAG  GACTGAACGGCGGGGGACTCAATACGAATTGA</p>
<p>SEQ ID NO: 25</p>	<p>2C3</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA  TTGGAGGGGCATCATTCAATTTTGCGGGAATT  AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG  GGGCAAGCTGTATGCGGTTGATTTTGGGACA  AGACAGGGACGAATTATAACAATGGCCCGGTA  TTATCACGATTTGTGCAAAAGGTTTGTAGACGA  AACGGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGCGGCGGAACACACTTTACTAC  ATAAAAAATTTGGATGGCGGTAATAAAATTGA  AAACGTCGTCACCATTGGTGGAGCAAACGGAC  TCGTTTCAAGCAGAGCATTACCAGGCACAGAT  CCAAATCAAATAATCTTTACACATCCGTCTA  TAGCTCAGCAGATCTTATTGTCGTCAACAGTC  TCTCTCGTTTAAATTGGCGCAAGAAACGTCAA  ATCCATGGCGTTGGACATATCGGTCTATTAAC  CTCAAGCCAAGTCAAAGGATATATTAAGAAG  GGCTTAACGGCGGGGGCCACAATACGAATTGA</p>
<p>SEQ ID NO: 26</p>	<p>2F11</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA  TCGGAGGAGCTTCATACAATTTTGCGGGAATT  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG  GGGCAAGCTGTATGCGGTTGATTTTGGGACA  AGACAGGGACGAATTATAACAATGGCCCGGTA  TTATCACGATTTGTGCAAAAGGTTTGTAGACGA  AACCGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGTGGCGGGAACACACTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAATTGA  AAACGTCGTAACGCTTGGCGGCGGAACCGTT  TGACGACAAGCAGGGCGCTTCCGGGAACAGAT</p>



		<p>CCAAATCAAAGATTTTATACACATCCATTTA                  CAGCAGTGCCGATATGATTGTCATGAATTACT                  TATCAAATTAGACGGTGCTAAAAACGTACAA                  ATTCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAAAGGATATATTAAGAAG                  GACTGAACGGCGGAGGCCATAATACGAATTGA</p>
SEQ ID NO: 27	KV11(6C7)	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TTGGAGGGGCATCATTTCAGTTTTCGGGAATT                  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG                  GGGCAAGCTGTATCCGGTTGATTTTGGGACA                  AGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCACGATTTGTGCAAAGGTTTGGACGA                  AACGGGTGCGAAAAAAGTGGATATTGTCGCTC                  ACAGTATGGGTGGCGCGAACACACTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAAATTGA                  AAACGTCGTAACGCTTGGCGGCGGAACCGTT                  TGACGACAAGCAAGGCGCTTCCGGGTACTGAT                  CCCAACCAAAGATCTTGTACACATCCGTTTA                  CAGTAGTGCTGATATGATTGTTATGAATTACT                  TATCAAATTAGACGGGGCTAAAAATGTTCAA                  ATTCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAAGAAG                  GACTGAACGGCGGGGGCCATAATACAAATTGA</p>
SEQ ID NO: 28	KV6(3A1)	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TTGGAGGGGCATCATTTCAGTTTTCGGGAATT                  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG                  GGGCAAGCTGTATGCGGTTGATTTTGGGACA                  AGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCACGATTTGTGCAAAGGTTTGGACGA                  AACGGGTGCGAAAAAAGTGGATATTGTCGCTC                  ACAGTATGGGTGGCGCGAACACACTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAAATTGA                  AAACGTCGTAACGCTTGGCGGCGGAACCGTT                  TGACGACAAGCAAGGCGCTTCCGGGTACTGAT                  CCCAACCAAAGATCTTGTACACATCCGTTTA                  CAGTAGTGCTGATATGATTGTTATGAATTACT                  TATCAAATTAGACGGGGCTAAAAATGTTCAA                  ATTCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAAGAAG                  GACTGAACGGCGGGGGCCATAATACAAATTGA</p>
SEQ ID NO: 29	KV2(2D1)	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TCGGAGGAGCTTCATACAGTTTTCGGGAATT                  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG                  GGGCAAGCTGTATGCGGTTGATTTTGGGACA                  AGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCACGATTTGTGCAAAGGTTTATAGACGA</p>

		<p>AACGGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGTGGCGCGAACACACTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAATTGA  AAACGTCGTAACGCTTGGCGGCGGAACCGTT  TGACGACAAGCAAGGCGCTTCCGGAACAGAT  CCCAACCAAAGATCTTGTACACATCCGTTTA  CAGTAGTGCCGATATGATTGTCATGAATTACT  TATCAAAATTAGACGGGGCTAAAAATGTTCAA  ATTCATGGTGTCGGACATATCGGCCTTCTGTA  CAGCAGCCAAGTCAACAGCCTGATTAAGAAG  GACTGAACGGCGGGGGCCAAAATACAAATTGA</p>
SEQ ID NO: 30	N2.5	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA  TCGGAGGAGCTTCATACAGTTTTCGGGAATT  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG  GGCAAGCTGTATGCGGTTGATTTTGGGACA  AGACAGGGACGAATTATAACAATGGCCCGGTA  TTATCACGATTTGTGCAAAAGTTTGTAGACGA  AACGGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGGGGCGCGAACACACTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAATTGA  AAACGTCGTAACGCTTGGCGGCGGAACCGTT  TGACGACAAGCAAGGCGCTTCCGGAACAGAT  CCCAACCAAAGATCTTGTACACATCCGTTTA  CAGTAGTGCTGATATGATTGTTATGAATTACT  TATCAAAATTAGACGGGGCTAAAAATGTTCAA  ATTCATGGCGTTGGGCACACTGGTTTATTGAT  GAACAGCCAAGTCAACAGCCTGATTAAGAAG  GACTGAACGGCGGGGGCCACAATACAAATTGA</p>
SEQ ID NO: 31	KV5(2H6)	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA  TTGGAGGAGCATCATACAATTTTCGGGAATT  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG  GGCAAGCTGTATACGGTTGATTTTGGGACA  AGACAGGCACAAATATAACAATGGCCCGGTA  TTATCACGATTTGTGCAAAAGTTTGTAGACGA  AACGGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGTGGCGCGAACACACTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAATTGA  AAACGTCGTAACGCTTGGCGGCGGAATCGTC  TTGTAACAGGCAAGGCGCTTCCGGAACAGAT  CCCAATCAAAGATTTTGTACGCATCCGTTTA  CAGCAGTGCCGATATGATTGTCATGAATTACT  TATCAAAATTAGACGGTGCTAAAAACGTTCAA  ATTCATGGCGTTGGGCACATTGGTTTATTGAT  GAACAGCCAAGTCAACAGCCTGATTAAGAAG  GACTGAACGGCGGGGGCCTGAATACAAATTGA</p>

<p>SEQ ID NO: 32</p>	<p>3E5</p>	<p>TGAACACAATCCAGTCGTTATGGTTCACGGTA                  TTGGAGGGGCATCATTC AATTTTGC GGG AATT                  AGGAGCTATCTCGTATCTCAGGGCTGGTCACG                  GGGCAAGCTGTATGCGGTTGATTTTGGGACA                  GGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCACGATTTGTGCAAAGGTTT TAGATGA                  AACCGGTGCGAAAAAGTGGACATTTGTCGCTC                  ACAGCATGGGTGGCGCGAACACACTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAAATTGA                  AAACGTCGTAACGCTTGGCGGCGGAACCGTT                  TGACGACAAGCAAGGCGCTTCCGGGAACAGAT                  CCAAATCAAAAGATTTTATACACATCCATTTA                  CAGCAGTGCCGATATGATTGTCATGAATTACT                  TATCAAAATTAGACGGGGCTAAAAATGTTCAA                  ATCCATGGCGTTGGACACATCGGCCCTTCTGTA                  CAGCAGCCAAGTCAACAGCCTGATTAAAGAAG                  GACTGAACGGCGGGGGCCTCAATACGAATTGA</p>
<p>SEQ ID NO: 33</p>	<p>G2.1</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TCGGAGGGGCATCATTC AATTTTGC GGG AATT                  AGGAGCTATCTCGTATCTCAGGGCTGGTCACG                  GGGCAAGCTGTATGCGGTTGATTTTGGGACA                  AGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCACGATTTGTGCAAAGGTTT TAGACGA                  AACCGGTGCGAAAAAGTGGACATTTGTCGCTC                  ACAGCATGGGCGGCGTAACACGCTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAAATTGA                  AAACGTCGTAACGCTTGGCGGCACGAACCGTT                  TGACGACAAGCAGGGCGCTTCCGGGAACAGAT                  CCAAATCAAAAGATTTTATACACATCCATTTA                  CAGCAGTGCCGATATGATTGTCATGAATTACT                  TATCAAAACTAGACGGTGCTAAAAACGTTCAA                  ATT CATGGCGTTGGGCACATTTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAAGAAG                  GACTGAACGGCGGGGGACTCAATACGAATTGA</p>
<p>SEQ ID NO: 34</p>	<p>3H24(G2)</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TTGGAGGGGCATCATTC AATTTTGC GGG AATT                  AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG                  GGACAAGCCGTATGCGGTTGATTTTGGGACA                  AGACAGGGACGAATTATAACAATGGCCCGGTA                  TTATCACGATTTGTGCAAAGGTTT TAGACAA                  AACGGGTGCGAAAAAGTGGATATTGTCGCTC                  ACAGCATGGGGGGCGGAACACACTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAAGTTGA                  AAACGTCGTAACGCTTGGCGGCGGAACCGTT                  TGACGACAAGCAAGGCGCTTCCGGGAACAGAT                  CCAAATCAAAAGATTTTATACACATCCATTTA</p>

		CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAAATTAGACGGTGCTAAAAACGTTCAA ATTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAAGAAG GACTGAACGGCGGGGGACTCAATACGAATTGA
SEQ ID NO: 35	KV10(4G6)	TGAACACAATCCAGTTGTTATGGTTCACGGTA TTGGAGGGGCATCATTCAATTTTGCGGGAATT AAGAGCTATCTCGTGTCTCAGGGCTGGCCGCG GGACAAGCTGTATGCAGTTGATTTTGGGACA AGACAGGGACGAATTATAACAATGGCCCCGTA TTATCACGATTTGTGCAAAAGGTTTATAGACGA AACGGGTGCGAAAAAAGTGGATATTGTCGCTC ACAGCATGGGTGGCGCGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAGTTGA AAGCGTCGTAACTTTGGCGGCGGAATCGTC TTGTAAACAGGCAAGGCGCTTCCGGAACTGAT CCCAACAAAAGATTTTATACACATCCATTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAAATTAGACGGTGCTAAAAACGTTCAA ATTCATGGCGTCGGACATATCGGCCTTCTGAT GAACAGCCAAGTCAACAGCCTGATTAAAGAAG GACTGAACGGCGGGGGCCACAATACAAAATTGA
SEQ ID NO: 36	KV12(6D4)	TGAACACAATCCAGTTGTTATGGTTCACGGTA TCGGAGGGGCATCATTTCAGTTTTCGGGAATT AGGAGCTATCTCGTATCTCAGGGCTGGCCGCG GGACAAGCTGTATGCGGTTGATTTTGGGACA AGACAGGCACAAATTATAACAATGGCCCCGTA TTATCACGATTTGTGCAAAAGGTATTAGATGA AACCGGTGCGAAAAAAGTGGATATTGTCGCC ACAGCATGGGTGGCGCGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAGTTGA AAACGTCGTGACGCTTGGCGGCGCCAACCGTT TGACGACAGGCAAGGCGCTTCCGGGTACTGAT CCCAATCAAAGATTTTATACACATCCGTTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAAATTAGACGGTGCTAAAAACGTTCAA ATTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGGCTGATTAAAGAAG GACTGAACGGCGGAGGCCACAATACAAAATTGA
SEQ ID NO: 37	N2.2	TGAACACAATCCAGTTGTTATGGTTCACGGTA TCGGAGGGGCATCATTTCAGTTTTCGGGAATT AGGAGCTATCTCGTATCTCAGGGCTGGCCGCG GGACAAGCTGTATGCGGTTGATTTTGGGACA AGACAGGCACAAATTATAACAATGGCCCCGTA TTATCACGATTTGTGCAAAAGGTATTAGATGA AACCGGTGCGAAAAAAGTGGATATTGTCGCC

		ACAGCATGGGTGGCGCGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAGTTGA AAACGTCGTGACGCTTGGCGGCGCCAACCGTT TGACGACAGGCAAGGCGCTTCCGGGTACTGAT CCCAATCAAAGATTTTATACACATCCGTTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAAATTAGACGGTGCTAAAAACGTTCAA ATTTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGGCTGATTAAGAAG GACTGAACGGCGGAGGCCACAATACAAATTGA
SEQ ID NO: 38	N2.3	TGAACACAATCCAGTTGTTATGGTTCACGGTA TCGGGGGGGCATCATTTCAGTTTTGCGGGAATT AGGAGCTATCTCGTATCTCAGGGCTGGCCGCG GGACAAGCTGTATGCGGTTGATTTTTGGGACA AGACAGGCACAAATTATAACAATGGCCCCGTA TTATCACGATTTGTGCAAAAGGTATTAGATGA AACC GG TGCGAAAAAGTGGATATTGTCGCC ACAGCATGGGTGGCGCGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAGTTGG AAACGTCGTGACGCTTGGCGGCGCCAACCGTT TGACGACAGGCAAGGCGCTTCCGGGTACTGAT CCCAATCAAAGATTTTATACACATCCGTTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAAATTAGACGGTGCTAAAAACGTTCAA ATTTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGGCTGATTAAGAAG GACTGAACGGCGGAGGCCACAATACAAATTGA
SEQ ID NO: 39	N2.1	TGAACACAATCCAGTTGTTATGGTTCACGGTA TCGGAGGGGCATCATTTCAGTTTTGCGGGAATT AGGAGCTATCTCGTATCCCAGGGCTGGCCGCG GGACAAGCTGTATGCGGTTGATTTTTGGGACA AGACAGGCACAAATTATAACAATGGCCCCGTA TTATCACGATTTGTGCAAAAGGTATTAGATGA AACC GG TGCGAAAAAGTGGATATTGTCGCC ACAGCATGGGTGGCGCGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAGTTGA AAACGTCGTGACGCTTGGCGGCGCCAACCGTT TGACGACAGGCAAGGCGCTTCCGGGTACTGAT CCCAATCAAAGATTTTATACACATCCGTTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAAATTAGACGGTGCTAAAAACGTTCAA ATTTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGGCTGATTAAGAAG GACTGAACGGCGGAGGCCACAATACAAATTGA
SEQ ID NO: 40	KV4(2E12)	TGAACACAATCCAGTTGTTATGGTTCACGGTA TTGGAGGGACATCATTCAATTTTTCGGGAAATT

		<p>AAGAGCTATCTCGTATCTCAGGGCTGGTCACG  GGACAAGCTGTATGCGGTTGATTTTTGGGACA  AGACAGGGACGAATTATAACAATGGCCCGGTA  TTATCACGATTTGTGCAAAAAGGTTTTAGACGA  AACGGGTGCGAAAAAAGTGGATATTGTGCGCTC  ACAGCATGGGCGGCGCCAACACGCTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAATTGA  AAACGTCGTGACGCTTGGCGGCGCGAACC GTT  TGACGACAAGCAAGGCGCTTCCGGGAACAGAT  CCAAATCAAAAAGATTTTATACACATCCATTTA  CAGCAGTGCCGATATGATTGTCATGAATTACT  TATCAAAATTAGACGGTGCTAAAAACGTTCAA  ATTCATGGCGTTGGGCACATTGGTTTTATTGAT  GAACAGCCAAGTCAACAGCCTGATTAAGAAG  GACTGAACGGCGGGGGCCACAATACAAATTGA</p>
<p>SEQ ID NO: 41</p>	<p>KV9(4C6)</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA  TCGGAGGGGCATCATTAGTTTTCGCGGAATF  AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG  GGACAAGCTGTATGCAGTTGATTTTAGTGACA  AAACAGGCACGAATTATAACAATGGCCCGGTA  TTATCACGATTTGTGCAAAAAGGTTTTAGACGA  AACGGGTGCGAAAAAAGTGGATATTGTGCGCTC  ACAGCATGGGGGGCGCGAACACACTTTACTAC  ATAAAAAATCTGGATGGCGGTAATAAAATTGA  AAACGTCGTAACACTTGGCGGCGCGAACC GTT  TGACGACAAGCAAGGCGCTTCCGGGTA CTGAT  CCCAACCAAAAAGATCTTGTACACATCCATTTA  CAGCAGTGCCGATATGGTTGTCATGAATTACT  TATCAAAATTAGACGGGGCTAAAAATGTTCAA  ATTCATGGTGTGCGGCACATTGGTTTTATTGAT  GAACAGCCAAGTCAACAGCCTGATTAAGAAG  GACTGAACGGCGGGGGCCACAATACGAATTGA</p>
<p>SEQ ID NO: 42</p>	<p>7D6</p>	<p>TAAACACAATCCAGTTGTTATGGTTCACGGTA  TTGGAGGGGCATCATACAATTTTCGCGGAATA  AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG  GGACAAGCTGTATGCAGTTGATTTTAGTGACA  AGACAGGGACGAATTATAACAATGGCCCGGTA  TTATCACGATTTGTGCAAAAAGGTTTTAGACGA  AACGGGTGCGAAAAAAGTGGATATTGTGCGCTC  ACAGCATGGGGGGCGCGAACACACTTTACTAC  ATAAAAAATCTGGACGGCGGTAATAAAATTGA  AAACGTCGTAACACTTGGCGGCGCGAACC GTT  TGACGACAAGCAAGGCGCTTCCGGGAACAGAT  CCAAATCAAAAAGATTTTATACACATCCATTTA  CAGCAGTGCCGATATGATTGTCATGAATTACT  TATCAAAACTAGACGGTGCTAAAAACGTTCAA</p>

		ATTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAAGAAG GACTGAACGGCGGGGGATTAAATACGAATTGA
SEQ ID NO: 43	3F3	TGAACACAATCCAGTTGTTATGGTTCACGGTA TTGGAGGGGCATCATTCAATTTTGCGGGAATT AAGAGCTATCTCGAATCTCAGGGCTGGTCACG GGGCAAGCTGTATGCGGTTGATTTTTGGGACA AGACCGGGACGAATTATAACAATGGCCCCGTA TTATCACGATTTGTGCAAAAAGGCTTTAGACGA AACGGGTGCGAAAAAAGTGGATATTGTGCGCTC ACAGCATGGGTGGCGCGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAATTGA AAACGTCGTAACGCTTGGCGGCGCGAACCGTT TGACGACAAGCAAGGCGCTTCCGGGAACAGAT CCAAATCAAAGATTTTATAACACATCCATTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAAATTAGACGGTGCTAAAAACGTTCAA ATCCATGGCGTTGGGCACATTTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAAGAAG GACTGAACGGCGGGGGCCAGAATACGAATTGA
SEQ ID NO: 44	2D11(G2)	TGAACACAATCCAGTTGTTATGGTTCACGGTA TCGGAGGGGCATCATTCAATTTTGCGGGAATT AAGAGCTATCTCGTATCTCAGGGCTGGTCACG GGGCAAGCTGTATGCGGTTGATTTTTGGGACA GGACAGGGACGAATTATAACAATGGCCCCGTA TTATCACGATTTGTGAAAAAGGTATTAGATGA AACCGGTGCGAAAAAAGTGGATATTGTGCGCTC ACAGCATGGGGGGCGCGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAATTGA AAACGTCGTCACACTTGGCGGCGCGAACCGTT CGACGACAAGCAAGGCGCTTCCGGGAACAGAT CCAAATCAAAGATTTTATAACACATCCATTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAAATTAGACGGTGCTAAAAACGTTCAA ATTCATGGCGTTGGGCACATTTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAAGAAG GGCTGAACGGCGGAGGCCAGAATACGAATTGA
SEQ ID NO: 45	3C23(G2)	TGAACACAATCCAGTTGTTATGGTTCACGGTA TTGGAGGGGCATCATTCAATTTTGCGGGAATT AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG GGGCAAGCTGTATGCGGTTGATTTTTGGGACA GGACAGGGACGAATTATAACAATGGCCCCGTA TTATCACGATTTGTGCAAAAAGGCTTTAGACGA AACGGGTGCGAAAAAAGTGGATATTGTGCGCTC ACAGCATGGGGGGCGCGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAATTGA

		AAACGTCGTCACACTTGGCGGCGCGAACC GTT CGACGACAAGCAAGGCGCTTCCGGGAACAGAT CCAAATCAAAGATTTTATACACATCCATTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAATTAGACGGTGCTAAAAACGTTCAA ATTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAGAAG GGCTTAACGGCGGGGGCCACAATACGAATTGA
SEQ ID NO: 46	G2.3	TGAACACAATCCAGTCGTTATGGTTCACGGTA TTGGAGGGGCATCATTCAATTTTGCGGGAATA AAGAGCTATCTCGTATCTCAGGGCTGGTCACG GGCAAGCTGTATGCGGTTGATTTTGGGACA GGACAGGGACGAATTATAACAATGGCCCCGTA TTATCACGATTTGTGCAAAGGTTTATAGACGA AACGGGTGCGAAAAAGTGGATATTGTCGCTC ACAGCATGGGTGGCGCGAACACACTTTACTAC ATAAAGAATCTGGACGGCGGAAATAAAATGTA AAACGTCGTAACGCTTGGCGGCGCGAACC GTT CGACGACAAGCAAGGCGCTTCCGGGAACAGAT CCAAATCAAAGATTTTATACACATCCATTTA CAGCAGTGCCGATATGATTGTCATGAATTGCT TATCAAATTAGACGGTGCTAAAAACGTTCAA ATTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAGAAG GACTGAACGGCGGGGGCCAGAATACGAATTGA
SEQ ID NO: 47	2A3	TGAACACAATCCAGTTGTTATGGTTCACGGTA TTGGAGGGGCATCGTTCAATTTTGCGGGAATT AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG GGACAAGCTGTATGCAGTTGATTTCAAAGACA AGACAGGGACGAATTATAACAATGGCCCCGTA TTATCACGATTTGTGAAAAAGTATTAGATGA AACGGGTGCGAAAAAGTGGATATTGTCGCTC ACAGCATGGGCGGCGCTAACACGCTTTACTAC ATAAAGAATCTGGACGGCGGAAATAAAATGTA AAACGTCGTAACGCTTGGCGGCGCGAACC GTT CGACGACAAGCAAGGCGCTTCCGGGTACTGAT CCCAACCAAAGATCTTGTACACATCCGTTTA CAGTAGTGCTGATATGATTGTTATGAATTACT TATCAAATTAGACGGTGCTAAAAACGTTCAA ATTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAGAAG GACTGAACGGCGGAGGCCATAATACAAATGTA
SEQ ID NO: 48	2F4	TGAACACAATCCAGTTGTTATGGTTCACGGTA TTGGAGGGGCATCATTCAATTTTGCGGGAATT AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG GGACAAGCTGTATGCGGTTGATTTTGGGACA



		<p>AGACAGGGACGAATTATAACAATGGCCCCGTA                  TTATCACGATTTGTGAAAAAGGTATTAGATGA                  AACCGGTGCGAAAAAAGTGGATATTGTGCGTC                  ACAGCATGGGTGGCGCTAACACGCTTTACTAC                  ATAAAAATCTGGACGGCGCGATAAAATTGA                  GAACGTCGTAACACTTGGCGGCGCGAACCGTT                  CGACGACAAGCAAGGCGCTTCCGGGAACAGAT                  CCAAATCAAAGATCTTGTACACATCCGTTTA                  CAGTAGTGCTGATATGATTGTCATGAATTACT                  TATCAAAATTAGACGGTGCTAAAAACGTTCAA                  ATTCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAGAAG                  GGCTGAACGGCGGAGGCCAGAATACGAATTGA</p>
<p>SEQ ID NO: 49</p>	<p>2B9(G2)</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TTGGAGGGGCATCATTCAATTTTGCGGGAATT                  AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG                  GGACAAGCTGTATGCAGTTGATTTTGGGGCA                  AGACAGGGACGAATTATAACAATGGCCCCGTA                  TTATCGCGTTTGTGAAAAAGGTATTAGATGA                  AACCGGTGCGAAAAAAGTGGATATTGTGCGTC                  ACAGCATGGGGGCGCGAACACACTTTACTAC                  ATAAAAATCTGGACGGCGGAAATAAAATTGA                  AAACGTCGTAACACTTGGCGGCGCGAACCGTT                  CGACGACAAGCAAGGCGCTTCCGGGAACAGAT                  CCAAATCAAAGATTTTATACACATCCATTTA                  CAGCAGTGCCGATATGATTGTCATGAATTACT                  TATCAAAATTAGACGGGGCTAAAAATGTTCAA                  ATTCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAGAAG                  GACTGAACGGCGGAGGCCAAAATACGAATTGA</p>
<p>SEQ ID NO: 50</p>	<p>2C5</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA                  TTGGAGGGGCATCATTCAATTTTGCGGGAATT                  AAGAGCTATCTCGTATCTCAGGGCTGGTCAGG                  GGGCAAGCTGTATGCAGTTGATTTTGGGACA                  AGACAGGGACGAATTATAACAATGGCCCCGTA                  TTATCGCGTTTGTGAAAAAGGTATTAGATGA                  AACCGGTGCGAAAAAAGTGGATATTGTGCGTC                  ACAGCATGGGCGGCGCTAACACGCTTTACTAC                  ATAAAAATCTGGATGGCGGTAATAAAATTGA                  AAACGTCGTCACACTTGGCGGCGCGAACCGTT                  CGACGACAAGCAAGGCGCTTCCGGGAACAGAT                  CCCAACCAAAGATTTTATACACATCCATTTA                  CAGCAGTGCCGATATGATTGTCATGAATTACT                  TATCAAAATTAGACGGTGCTAAAAACGTTCAA                  ATTCATGGCGTTGGGCACATTGGTTTATTGAT                  GAACAGCCAAGTCAACAGCCTGATTAAGAAG</p>

<p>SEQ ID NO: 51</p>	<p>KV1(2A6)</p>	<p>GACTGAACGGCGGAGGCCAAAATACGAATTGA  TGAACACAATCCAGTTGTTATGGTTCACGGTA  TTGGAGGGGCATCATTCAATTTTGCGGGAATT  AAGAGCTATCTCGTATCTCAGGGCTGGTCACG  GGGCAAGCTGTATGCGGTTGATTTCAAGGACA  AGACAGGCACAAATATAACAATGGCCCCGTA  TTATCACGATTTGTGAAAAAGGTATTAGATGA  AACCGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGCGGCGCTAACACGCTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAATTGA  AAACGTCGTAACGCTTGGCGGCGCGAACC GTT  CGACGACAAGCAAGGCGCTTCCGGGTACTGAT  CCCAACC AAAAGATTTTATACACATCCATTTA  CAGCAGTGCCGATATGATTGTCATGAATTACT  TATCAAAATTAGACGGTGCTAAAAACGTTCAA  ATTCATGGCGTTGGGCACATTGGTTTATTGAT  GAACAGCCAAGTCAACAGCCTGATTAAGAAG  GGCTTAACGGCGGGGGCCAGAATACGAATTGA</p>
<p>SEQ ID NO: 52</p>	<p>2D13(G2)</p>	<p>TAAACACAATCCAGTTGTTATGGTTCACGGTA  TTGGAGGGGCATCATTCAATTTTGCGGGAATT  AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG  GGACGAGCTGTATGCGGTTGATTTTGGGACG  AGACAGGGACGAATTATAACAATGGCCCCGTA  TTATCACGATTTGTGCAAAGGTTT TAGACGA  AACCGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGTGGCGCGAACACACTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAATTGA  AAACGTCGTAACGCTTGGCGGCGCGAACC GTT  CGACGACAAGCAAGGCGCTTCCGGGTACAGAT  CCAAATCAAAGATTTTATACACATCCATTTA  CAGCAGTGCCGATATGATTGTCATGAATTACT  TATCAAAATTAGACGGTGCTAAAAATGTTCAA  ATTCATGGCGTTGGGCACATTGGTTTATTGAT  GAACAGCCAAGTCAACAGCCTGATTAAGAAG  GGCTGAACGGCGGAGGCCAAAATACGAATTGA</p>
<p>SEQ ID NO: 53</p>	<p>3C8</p>	<p>TGAACACAATCCAGTTGTTATGGTTCACGGTA  TCGGAGGGGCATCATTCAATTTTGCGGGAATT  AAGAGCTATCTCGTATCTCAGGGCTGGTCGCG  GGACAAGCTGTATGCGGTTGATTTTGGGACA  AGACAGGGACGAATTATAACAATGGCCCCGTA  TTATCACGATTTGTGCAAAGGTTT TAGACGA  AACCGGTGCGAAAAAAGTGGATATTGTCGCTC  ACAGCATGGGTGGCGCGAACACACTTTACTAC  ATAAAAAATCTGGACGGCGGAAATAAAGTTGA  AAACGTCGTAACACTTGGCGGCGCGAATCGTT  CGACGACAAGCAAGGCGCTTCCGGGAACAGAT</p>

		CCAAATCAAAGATTTTATACACATCCATTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAATTAGACGGTGCTAAAAACGTTCAA ATTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAAGAAG GACTGAACGGCGGGGGCCAAAATACAAATTGA
SEQ ID NO: 54	2D5	TGAACACAATCCAGTTGTTATGGTTCACGGTA TCGGAGGGGCATCATTCAATTTGCGGGAATT AAGAGCTATCTCGTATCTCAGGGCTGGTCACG GGCAAGCTGTATGCGGTTGATTTTTGGGACA AGACAGGGACGAATTATAACAATGGCCCCGTA TTATCAGATTGTGCAAAAGGTTTAGACGA AACGGGTGCGAAAAAGTGGATATTGTCGCTC ACAGCATGGGTGGCGGAACACACTTTACTAC ATAAAAAATCTGGACGGCGGAAATAAAATTGA AAACGTCGTAACACTTGGCGGCGGAACCGTT CGACGACAAGCAAGGCGCTTCCGGGAACAGAT CCAAATCAAAGATTTTATACACATCCATTTA CAGCAGTGCCGATATGATTGTCATGAATTACT TATCAAATTAGACGGTGCTAAAAATGTTCAA ATTCATGGCGTTGGGCACATTGGTTTATTGAT GAACAGCCAAGTCAACAGCCTGATTAAAGAAG GGCTGAACGGCGGAGGACAAAATACAAATTGA
SEQ ID NO: 55	405 (pumilus)	MKFVKRRI IALVTILVLSVTSLFAMQPSAKAA EHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLRFVQKVLDE TGAKKVDIVAHSMGGANTPYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNAQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 56	406 (subtilis)	MKFVKRRI IALVTILMLSVTSLFALQPSAKAA EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKLYAVDFWDKTGTNYNNGPVLPRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKVA NVVTLGGANRLTTGKALPGTDPNQKILYTSIY SSADMIVINYLSRLDGARNVQIHGVGHIGLLY SSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 57	402 (megaterium)	MKFVKRRI IALVTILVLSVTSLFAMQPSAKAA DTIQLLWFTGIGGASYNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 58	400 (lentus)	MKFVKRRI IALVTILVLSVTSLFAMQPSAKAA EHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR

		GKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 59	396 (circulans)	MKFIKRRIIALVTILVLSVTSLFAMQPSAKAA EHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 60	392 (azotoformans)	MKFFVKRRIIALVTILVLSVTSLFAMQPSAKAA EHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR GELYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSANMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGGLDTN
SEQ ID NO: 61	398 (firmus)	MKFFVKRRIIALVTILVLSVTSLFAMQPSAKAA EHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNAQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 62	393 (badius)	MKFFVKRRIIALVTILVLSVTSLFAMQPSAKAA EHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 63	Dc5h	MKFFVKRRIIALVTILMLSVTSLFALQPSAKAA EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKLYAVDFKDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKVE NVVTLGGANRLTTGKALPGTDPNQKILYTSIY SSADMIVMNYLSRLDGARNVQIHGVGHIGLLY SSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 64	Dc5f	MKFFVKRRIIALVTILMLSVTSLFALQPSAKAA EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKLYAVDFXDKTGNRRNNGPRLSRFVKDVLDK TGAKKVDIVAHSMGGANTLYYIKNLDGGDKIE NVVTIGGANLVSSRALPGTDPNQKILYTSVY SSADLIVVNSLSRLIGARNILIHGVGHIGLLT

		SSQVKGYIKEGLNGGGLNTN
SEQ ID NO: 65	Dc5c1	MKVIFVKKRSLQILVALALVLSIAFIQPKEA KAAEHNPVVMVHGMGGASYNFASIKRYLVSQG WDQNQLFAIDFIDKTGNLNNNGPRLSRFVKDV LAKTGAKKVDIVAHSMGGANTLYYIKNLDGGD KIENVVTLGGANGLVSLRALPGTDPNQKILYT SVYSSADLIVVNSLSRLIGARNVLIHGVGHIG LLTSSQVKGYVKEGLNGGGQNTN
SEQ ID NO: 66	Dc5a2	MKVIFVKKRSLQILVVLALVMGSMAFIQPKEI RAAEHNPVVMVHGMGGASYNFASIKSYLVSQG WDRNQLFAIDFIDKTGNRNNNGPRLSRFVKDV LAKTGAKKVDIVAHSMGGANTLYYIKNLDGGD KIENVVTLGGANGLVSLRALPGTDPNQKILYT SVYSSADLIVVNSLSRLIGARNVLIHGVGHIG LLASSQVKGYIKEGLNGGGQNTN
SEQ ID NO: 67	Dc512	MKVIFVKKRSLQILLIALALVIGSMAFIQPKEA KAAEHNPVVMVHGI GGASYNFFSIKSYLATQG WDRNQLYAIDFIDKTGNRNNNGPRLSRFVKDV LDKTGAKKVDIVAHSMGGANTLYYIKNLDGGD KIENVVTIGGANGLVSSRALPGTDPNQKILYT SVYSSADLIVVNSLSQFNWRKKHPDPGVGHIG LLTSSQVKGYIKEGLNGGGLNTN
SEQ ID NO: 68	Sga	MKFVKRRIIALVITILMLSVTSLFALQPSAKAA EHNPVVMVHGI GGASFNFAGIKSYLVSQGWSR DKLYAVDFRDKTGNLNNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLVTGKALPGTDPNQKILYTSVY SSADMIVMNYLTKLDGAKNVQIHGVGHIGLLY SSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 69	Sgc	MKFVKRRIIALVITILMLSVTSLFALQPSAKAA EHNPVVMVHGI GGASFNFAGIKSYLVSQGWSR DKLYAVDFWDKTGNLNNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLVTGKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLY SSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 70	Sgd	MKFVKRRIIALVITILMLSVTSLFALQPSAKAA EHNPVVMVHGI GGASFNFAGIKSYLVSQGWSR DKLYAVDFSDKTGNLNNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLVTGKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLY SSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 71	Sgf	MKFVKRRIIALVITILMLSVTSLFALQPSAKAA EHNPVVMVHGI GGASFNFAGIKSYLVSQGWSR DKLYAVDFKDKTGNRNNNGPRLSRFVKDVLDK

		TGAKKVDIVAHSMGGANTLYYIKNLDGGDKIE NVVTIGGANGLVSSRALPGTDPNQKILYTSVY SSADLIVVNSLSRLIGARNVQIHGVGHIGLLT SSQVKGYIKEGLNGGGLNTN
SEQ ID NO: 72	Sgh	MKFKVRRILALVTILMLSVTSLFALQPSAKAA EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKLYAVDFIDKTGNRRNNGPRLSRFVKDVLDK TGAKKVDIVAHSMGGANTLYYIKNLDGGDKIE NVVTIGGANGLVSSRALPGTDPNQKILYTSVY SSADLIVVNSLSRLIGARNVQIHGVGHIGLLT SSLVKGYIKEGLNGGQNTN
SEQ ID NO: 73	Mt2b1	MKVIFVKRSLQILVALALVIGSMAFIQPKIE KAAEHNPVVMVHGIGGASYNFASIKSYLVNQG WDRNQLFAIDFIDKTGNRRNNGPRLSRFVKDV LDKTGAKKVDIVAHSMGGANTLYYIKNLDGGD KIENVVTIGGANGLVSLRALPGTDPNQKILYT SVYSSADLIVVNSLSRLTGARNVLIHGVGHIG LLTSSQVKGYIKEGLNGGGLNTN
SEQ ID NO: 74	H2a	MKFKVRRILALVTILMLSVTSLFALQPSAKAA EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKLYAVDFRDKTGNRRNNGPRLSKFVKDVLDK TGAKKVDIVAHSMGGANTLYYIKNLDGGDKIE NVVTIGGANGLVSSRALPGTDPNQKILYTSVY KLSRSHCRQOQLSFNWLQETVQIHGVGHIGLL TSSQVKGYIKEGLNGGGLNTN
SEQ ID NO: 75	1f15(G2)	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKVE NVVTLGGTNRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 76	3C12	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVKDVLDK TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 77	3N19(G2)	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDRTGTNYNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY GSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 78	G2.2	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE

		NVVTLGGANRSTTSKALPGTDPNQKILYTSIY GSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 79	2C3	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLRSRVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTIGGANLVSSRALPGTDPNQKILYTSVY SSADLIVVNSLSRLIGARNVQIHGVGHIGLLT SSQVKGYIKEGLNGGGHNTN
SEQ ID NO: 80	2F11	EHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLRSRVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSRALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVKGYIKEGLNGGGLNTN
SEQ ID NO: 81	KV11(6C7)	EHNPVVMVHGIGGASFSFAGIKSYLVSQGWSR GKLYPVDFWDKTGTNYNNGPVLRSRVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 82	KV6(3A1)	EHNPVVMVHGIGGASFSFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLRSRVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 83	KV2(2D1)	EHNPVVMVHGIGGASYSFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLRSRVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLY SSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 84	N2.5	EHNPVVMVHGIGGASYSFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLRSRVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 85	KV5(2H6)	EHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR GKLYTVDFWDKTGTNYNNGPVLRSRVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLVTGKALPGTDPNQKILYASVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN

SEQ ID NO: 86	3E5	EHNPVVMVHGIGGASFNFAGIRSYLVSQGWSR GKLYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVD IVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLY SSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 87	G2.1	EHNPVVMVHGIGGASFNFAGIRSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVD IVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGTNRLTTSRALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 88	3H24(G2)	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKPYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVD IVAHSMGGANTLYYIKNLDGGNKVE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGLNTN
SEQ ID NO: 89	KV10(4G6)	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWPR DKLYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVD IVAHSMGGANTLYYIKNLDGGNKVE SVVTLGGANRLVTGKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 90	KV12(6D4)	EHNPVVMVHGIGGASFSFAGIRSYLVSQGWPR DKLYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVD IVAHSMGGANTLYYIKNLDGGNKVE NVVTLGGANRLTTGKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNRLIKEGLNGGGHNTN
SEQ ID NO: 91	N2.2	EHNPVVMVHGIGGASFSFAGIRSYLVSQGWPR DKLYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVD IVAYSMMGGANTLYYIKNLDGGNKVE NVVTLGGANRLTTGKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNRLIKEGLNGGGHNTN
SEQ ID NO: 92	N2.3	EHNPVVMVHGIGGASFSFAGIRSYLVSQGWPR DKLYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVD IVAHSMGGANTLYYIKNLDGGNKVG NVVTLGGANRLTTGKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNRLIKEGLNGGGHNTN
SEQ ID NO: 93	N2.1	EHNPVVMVHGIGGASFSFAGIRSYLVSQGWPR DKLYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVD IVAHSMGGANTLYYIKNLDGGNKVE NVVTLGGANRLTTGKALPGTDPNQKILYTSVY



		SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNRLIKEGLNGGGHNTN
SEQ ID NO: 94	KV4(2E12)	EHNPVVMVHGIGGTSFNFAGIKSYLVSQGWSR DKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 95	KV9(4C6)	EHNPVVMVHGIGGASFSFAGIKSYLVSQGWSR DKLYAVDFSDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 96	7D6	KHNPVVMVHGIGGASYNFAGIKSYLVSQGWSR DKLYAVDFSDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 97	3F3	EHNPVVMVHGIGGASFNFAGIKSYLESQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVQKALDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRLTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 98	2D11(G2)	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDRGTGTNYNNGPVLSRFVKKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 99	3C23(G2)	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGHNTN
SEQ ID NO: 100	G2.3	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDRGTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMSGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNCLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 101	2A3	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR

		DKLYAVDFKDKTGTNYNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 102	2F4	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKLYAVDFWDKTGTNYNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGDKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSVY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 103	2B9(G2)	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKLYAVDFWGTGTNYNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 104	2C5	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 105	KV1(2A6)	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFKDKTGTNYNNGPVLSRFVKKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 106	2D13(G2)	KHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DELYAVDFWDETGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 107	3C8	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR DKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKVE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM NSQVNSLIKEGLNGGGQNTN
SEQ ID NO: 108	2D5	EHNPVVMVHGIGGASFNFAGIKSYLVSQGWSR GKLYAVDFWDKTGTNYNNGPVLSRFVQKVLDE TGAKKVDIVAHSMGGANTLYYIKNLDGGNKIE NVVTLGGANRSTTSKALPGTDPNQKILYTSIY SSADMIVMNYLSKLDGAKNVQIHGVGHIGLLM

	NSQVNSLIKEGLNGGGQNTN
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WHAT IS CLAIMED IS:

1. An isolated or recombinant polypeptide comprising a sequence having at least 97% amino acid sequence identity to any one of SEQ ID NO: 75 to SEQ ID NO: 108.
2. The polypeptide of claim 1, wherein said polypeptide comprises lipase activity.
3. The polypeptide of claim 1, wherein said polypeptide degrades geranyl butyrate, neryl butyrate, or both geranyl butyrate and neryl butyrate.
4. The polypeptide of claim 3, wherein said polypeptide exhibits enantioselectivity for geranyl butyrate over neryl butyrate.
5. The polypeptide of claim 4, comprising a sequence selected from: SEQ ID NO:76, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:86, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:78, SEQ ID NO:87, SEQ ID NO:100, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:88, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:103, or SEQ ID NO:106.
6. The polypeptide of claim 3, wherein said polypeptide exhibits enantioselectivity for neryl butyrate over geranyl butyrate.
7. The polypeptide of claim 6, comprising a sequence selected from: SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:105, SEQ ID NO:84, SEQ ID NO:91, SEQ ID NO:92, or SEQ ID NO:93.
8. The polypeptide of claim 1, comprising a polypeptide encoded by a polynucleotide sequence, which polynucleotide sequence hybridizes under highly stringent conditions over substantially the entire length of: a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54, or a complementary sequence thereof; or a polynucleotide sequence encoding a polypeptide sequence selected from SEQ ID NO: 55 to SEQ ID NO:

108, or a complementary sequence thereof; wherein said polypeptide comprises one or more of: Lys at position 1; Thr at position 14; Ser at position 17; Arg at position 22; Glu at position 26; Pro at position 31; Gly at position 33; Glu at position 34; Pro at position 35; Pro or Thr at position 37; Ser or Lys at position 41; Gly at position 42; Arg or Glu at position 43; 5 Ala at position 61; Tyr at position 75; Gly at position 96; Ser at position 97; Thr at position 104; Ser at position 107; Ala at position 125; Gly at position 129; Val at position 134; Cys at position 138; Lys at position 141; Lys at position 146; Thr at position 156; Met at position 160; Arg at position 166; or His at position 177.

9. The polypeptide of claim 1 comprising one or more of: Lys at position 10 1; Thr at position 14; Ser at position 17; Arg at position 22; Glu at position 26; Pro at position 31; Gly at position 33; Glu at position 34; Pro at position 35; Pro or Thr at position 37; Ser or Lys at position 41; Gly at position 42; Arg or Glu at position 43; Ala at position 61; Tyr at position 75; Gly at position 96; Ser at position 97; Thr at position 104; Ser at position 107; Ala at position 125; Gly at position 129; Val at position 134; Cys at position 15 138; Lys at position 141; Lys at position 146; Thr at position 156; Met at position 160; Arg at position 166; or His at position 177.

10. The polypeptide of claim 9, wherein said polypeptide has lipase activity.

11. The polypeptide of claim 9, wherein said polypeptide degrades geranyl butyrate, neryl butyrate, or both geranyl butyrate and neryl butyrate.

12. The polypeptide of claim 11, wherein said polypeptide exhibits 20 enantioselectivity for geranyl butyrate over neryl butyrate.

13. The polypeptide of claim 12, comprising one or more of: Arg at position 22; Gly at position 33; Ser or Lys at position 41; Arg at position 43; Ser at position 107; Lys at position 141; Lys at position 146; Met at position 160; or His at position 177.

14. The polypeptide of claim 12, comprising one or more of: Arg at position 25 43; or Ser at position 107.

15. The polypeptide of claim 11, wherein said polypeptide exhibits enantioselectivity for neryl butyrate over geranyl butyrate.

16. The polypeptide of claim 15, comprising one or more of: Ser at position 17; Arg at position 22; Pro at position 31; Gly at position 33; Ser or Lys at position 41; Lys at position 141; Lys at position 146; Met at position 160; Arg at position 166; or His at position 177.

17. The polypeptide of claim 15, comprising one or more of: Ser at position 17; Pro at position 31; or Arg at position 166.

18. An isolated or recombinant polypeptide comprising a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 55, 61, 64, 65, 67, 68, 70 or 72.

19. The polypeptide of claim 18, wherein said polypeptide comprises a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 55.

20. The polypeptide of claim 19, wherein said polypeptide comprises a sequence selected from SEQ ID NO: 55, 58-62, 75-78, 80-88, or 94-108, or the mature region thereof.

21. The polypeptide of claim 18, wherein said polypeptide comprises a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 61.

22. The polypeptide of claim 21, wherein said polypeptide comprises a sequence selected from SEQ ID NO: 55, 57-62, 75-78, 80-90, or 93-108, or the mature region thereof.

23. The polypeptide of claim 18, wherein said polypeptide comprises a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 64.

24. The polypeptide of claim 23, wherein said polypeptide comprises a sequence selected from SEQ ID NO: 64, 71, or 72, or the mature region thereof.

25. The polypeptide of claim 18, wherein said polypeptide comprises a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID  
5 NO: 65.

26. The polypeptide of claim 25, wherein said polypeptide comprises a sequence selected from SEQ ID NO: 65, 66, or 73, or the mature region thereof.

27. The polypeptide of claim 18, wherein said polypeptide comprises a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID  
10 NO: 67.

28. The polypeptide of claim 27, wherein said polypeptide comprises the sequence SEQ ID NO: 67, or the mature region thereof.

29. The polypeptide of claim 18, wherein said polypeptide comprises a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID  
15 NO: 68.

30. The polypeptide of claim 29, wherein said polypeptide comprises a sequence selected from SEQ ID NO: 68 or 101, or the mature region thereof.

31. The polypeptide of claim 18, wherein said polypeptide comprises a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID  
20 NO: 70.

32. The polypeptide of claim 31, wherein said polypeptide comprises a sequence selected from SEQ ID NO: 63, 68-70, 82-83, 85-86, 96, or 101-102, or the mature region thereof.

33. The polypeptide of claim 18, wherein said polypeptide comprises a sequence having at least 94% amino acid sequence identity to the mature region of SEQ ID NO: 72.

5 34. The polypeptide of claim 33, wherein said polypeptide comprises a sequence selected from SEQ ID NO: 64, 71, or 72, or the mature region thereof.

35. An isolated or recombinant polypeptide comprising a sequence having at least 85% amino acid sequence identity to the mature region of SEQ ID NO: 74.

36. The polypeptide of claim 35, wherein said polypeptide comprises a sequence selected from SEQ ID NO: 63, 71-72, 74, or 79.

10 37. An isolated or recombinant polypeptide comprising a sequence having at least 99% amino acid sequence identity to the mature region of SEQ ID NO: 56.

38. An isolated or recombinant polypeptide exhibiting enantioselective lipase activity, which polypeptide comprises an amino acid sequence of any one of SEQ ID NO: 55 through SEQ ID NO: 108.

15 39. An isolated or recombinant polypeptide exhibiting enantioselective lipase activity, which polypeptide comprises at least 45 contiguous amino acid residues of a polypeptide encoded by a coding polynucleotide sequence, the coding polynucleotide sequence selected from the group consisting of:

20 (a) a polynucleotide sequence selected from any of SEQ ID NO: 1 to SEQ ID NO: 54;

(b) a polynucleotide sequence that encodes a polypeptide selected from any of SEQ ID NO: 55 to SEQ ID NO: 108; and,

25 (c) a polynucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a polynucleotide sequence (a) or (b), or which hybridizes to a subsequence comprising at least 100 nucleotides thereof, wherein the polynucleotide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769,



AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874,  
AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273,  
CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662,  
CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957,  
5 S23934, U78785, X95309, Z99105, and Z99108.

40. The isolated or recombinant polypeptide of claim 38, wherein the polypeptide is enantioselective for either a *cis* form substrate enantiomer or for a *trans* form substrate enantiomer.

41. The isolated or recombinant polypeptide of claim 40, wherein the  
10 polypeptide comprises an enantiomeric ratio of at least 2 for the *cis* form substrate enantiomer or for the *trans* form substrate enantiomer.

42. The isolated or recombinant polypeptide of claim 40, wherein the polypeptide comprises an enantiomeric ratio of at least 5 for the *cis* form substrate enantiomer or for the *trans* form substrate enantiomer.

43. The isolated or recombinant polypeptide of claim 40, wherein the  
15 polypeptide comprises an enantiomeric ratio of at least 10 for the *cis* form substrate enantiomer or for the *trans* form substrate enantiomer.

44. The isolated or recombinant polypeptide of claim 40, wherein the  
20 polypeptide comprises an enantiomeric ratio of at least 50 for the *cis* form substrate enantiomer or for the *trans* form substrate enantiomer.

45. The isolated or recombinant polypeptide of claim 40, wherein the polypeptide comprises an enantiomeric ratio of at least 100 for the *cis* form substrate enantiomer or for the *trans* form substrate enantiomer.

46. An isolated or recombinant polypeptide which is at least 99% or more  
25 identical over a comparison window of 45 contiguous amino acids to one or more of SEQ ID NO: 55 to SEQ ID NO: 108.

47. An isolated or recombinant polypeptide encoded by a nucleic acid comprising a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54, or a complementary polynucleotide sequence thereof;

5 (b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108, or a complementary polynucleotide sequence thereof;

(c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b), or which hybridizes to a subsequence thereof comprising at least 100 residues thereof, wherein the  
10 polynucleotide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662,  
15 CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108;

(d) a polynucleotide sequence comprising all or a fragment of (a), (b), or (c), wherein the fragment encodes a polypeptide comprising lipase activity; and,

(e) a polynucleotide sequence encoding a polypeptide, the polypeptide  
20 comprising an amino acid sequence which is substantially identical over at least 45 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108, wherein the polynucleotide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874,  
25 AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

48. A polynucleotide sequence encoding a polypeptide comprising lipase activity produced by mutating or recombining one or more polynucleotide sequence of claim 47.

5 49. The isolated or recombinant polypeptide of claim 47, the polypeptide comprising an amino acid sequence of any one of SEQ ID NO: 55 to SEQ ID NO: 108.

50. The isolated or recombinant polypeptide of claim 47, wherein the encoded polypeptide exhibits lipase activity.

51. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits enantioselective lipase activity.

10 52. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits lipase activity with respect to tributyrin.

53. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits lipase activity with respect to tributyrin in DMF.

15 54. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits lipase activity with respect to tributyrin after heat treatment.

55. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits enantioselective lipase activity with respect to neryl- butyrate.

20 56. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits enantioselective lipase activity with respect to geranyl- butyrate.

57. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits lipase activity with respect to methyl esters.

58. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits lipase activity with respect to pentadecanolide.

59. The isolated or recombinant polypeptide of claim 50, wherein the encoded polypeptide exhibits lipase activity with respect to oxacyclotridecan.

60. An isolated or recombinant polypeptide comprising at least 45 contiguous amino acid residues of any of the polypeptides of claim 47, wherein the polypeptide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

61. The isolated or recombinant polypeptide of claim 60, which is substantially identical over at least 180 contiguous amino acids of the encoded polypeptide, wherein the polypeptide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

62. The isolated or recombinant polypeptide of claim 60, which is substantially identical over at least 212 amino acids of the encoded polypeptide, wherein the polypeptide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

63. The isolated or recombinant polypeptide of claim 60, which is substantially identical over at least 213 amino acids of the encoded polypeptide, wherein the polypeptide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

64. The isolated or recombinant polypeptide of claim 60, which is substantially identical over at least 215 amino acids of the encoded polypeptide, wherein the polypeptide sequence does not comprise a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

65. The polypeptide of claim 1, 18, 35, 36, or 47, comprising a leader sequence.

66. The polypeptide of claim 1, 18, 35, 36, or 47, comprising a precursor polypeptide.

67. The polypeptide of claim 1, 18, 35, 36, or 47, wherein the polypeptide comprises a secretion signal or a localization signal.

68. The polypeptide of claim 1, 18, 35, 36, or 47, wherein the polypeptide comprises an epitope tag.

69. The polypeptide of claim 1, 18, 35, 36, or 47, wherein the polypeptide comprises a fusion protein comprising one or more additional amino acid sequences.

70. The polypeptide of claim 1, 18, 35, 36, or 47, further comprising a polypeptide purification subsequence.

5 71. The polypeptide of claim 70, wherein the polypeptide purification subsequence is selected from the group consisting of: an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion.

72. The polypeptide of claim 1, 18, 35, 36, or 47, further comprising a methionine residue at the N-terminus.

10 73. The polypeptide of claim 1, 18, 35, 36, or 47, wherein the polypeptide further comprises a modified amino acid.

74. The polypeptide of claim 73, wherein the modified amino acid is selected from the group consisting of: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

15 75. A composition comprising one or more polypeptide of claim 73 and a pharmaceutically acceptable excipient.

76. A composition comprising one or more polypeptide of claim 1, 18, 35, 36, or 47, and a pharmaceutically acceptable excipient.

20 77. A polypeptide which comprises a unique subsequence in a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108, wherein the unique subsequence is unique as compared to a polypeptide sequence corresponding to an amino acid sequence or encoded by a nucleic acid sequence corresponding to any of GenBank accession numbers:  
25 I16WA, I16WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274,

CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

5           78. A polypeptide which is specifically bound by a polyclonal antisera raised  
against at least one antigen, which at least one antigen comprises at least one amino acid  
sequence of SEQ ID NO: 55 to SEQ ID NO: 108, or a fragment thereof, wherein the antisera  
is subtracted with a polypeptide sequence corresponding to an amino acid sequence or  
encoded by a nucleic acid sequence corresponding to any of GenBank accession numbers:  
116WA, 116WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257,  
10   AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623,  
AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274,  
CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850,  
D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785,  
X95309, Z99105, and Z99108.

15           79. An antibody or antisera produced by administering the polypeptide of  
claim 1, 18, 35, 36, or 47 to a mammal, which antibody or antisera specifically binds at least  
one antigen, said at least one antigen comprising a polypeptide comprising any one of the  
amino acid sequences of SEQ ID NO: 55 to SEQ ID NO: 108, which antibody or antisera  
does not specifically bind to a peptide encoded by a nucleic acid corresponding to one or  
20   more of GenBank accession numbers: 116WA, 116WB, A02813, A02815, A34992,  
AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617,  
AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967,  
C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664,  
CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068,  
25   M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

80. An antibody or antisera which specifically binds a polypeptide, the  
polypeptide comprising an amino acid sequence selected from the group consisting of SEQ  
ID NO: 55 to SEQ ID NO: 108, wherein the antibody does not specifically bind to a peptide  
encoded by a nucleic acid corresponding to one or more of GenBank accession numbers:

1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257,  
 AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623,  
 AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274,  
 CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850,  
 5 D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785,  
 X95309, Z99105, and Z99108.

81. An isolated or recombinant nucleic acid comprising: a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO:  
 10 54, or a complementary polynucleotide sequence thereof;

(b) a polynucleotide sequence encoding a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108, or a complementary polynucleotide sequence thereof;

(c) a polynucleotide sequence which hybridizes under highly stringent conditions over substantially the entire length of polynucleotide sequence (a) or (b), or which  
 15 hybridizes to a subsequence thereof comprising at least 100 residues, wherein the polynucleotide subsequence is unique as compared to a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652,  
 20 CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108; and,

(d) a polynucleotide sequence comprising all or a fragment of (a), (b), or (c), wherein the fragment encodes a polypeptide comprising lipase activity, and wherein the  
 25 polynucleotide subsequence is unique as compared to a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971,



CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

82. An isolated or recombinant nucleic acid comprising a polynucleotide sequence encoding a polypeptide, the polypeptide comprising an amino acid sequence which  
5 is substantially identical over at least 45 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108, wherein the polynucleotide subsequence is unique as compared to a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356,  
10 BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

83. The isolated or recombinant nucleic acid of claim 82, wherein the  
15 polypeptide comprises an amino acid sequence which is substantially identical over at least 45 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108, wherein the polynucleotide subsequence is unique as compared to a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617,  
20 AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

84. The isolated or recombinant nucleic acid of claim 82, wherein the  
25 polypeptide comprises an amino acid sequence which is substantially identical over at least 180 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108, wherein the polynucleotide subsequence is unique as compared to a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617,

AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

5                   **85.** The isolated or recombinant nucleic acid of claim 82, wherein the polypeptide comprises an amino acid sequence which is substantially identical over at least 212 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108, wherein the polynucleotide subsequence is unique as compared to a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992,  
10   AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

15                   **86.** The isolated or recombinant nucleic acid of claim 82, wherein the polypeptide comprises an amino acid sequence which is substantially identical over at least 213 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108, wherein the polynucleotide subsequence is unique as compared to a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992,  
20   AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

25                   **87.** The isolated or recombinant nucleic acid of claim 82, wherein the polypeptide comprises an amino acid sequence which is substantially identical over at least 215 contiguous amino acid residues of any one of SEQ ID NO: 55 to SEQ ID NO: 108, wherein the polynucleotide subsequence is unique as compared to a sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992,

AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617,  
AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967,  
C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664,  
CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068,  
5 M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

88. The nucleic acid of claim 81 or 82, wherein the encoded polypeptide exhibits lipase activity.

89. The nucleic acid of claim 81 or 82, wherein the encoded polypeptide exhibits enantioselective lipase activity.

10 90. The nucleic acid of claim 88, wherein the encoded polypeptide exhibits lipase activity with respect to tributyrin.

91. The nucleic acid of claim 88, wherein the encoded polypeptide exhibits lipase activity with respect to tributyrin in DMF.

15 92. The nucleic acid of claim 88, wherein the encoded polypeptide exhibits lipase activity with respect to tributyrin after heat treatment.

93. The nucleic acid of claim 89, wherein the encoded polypeptide exhibits enantioselective lipase activity with respect against neryl-butyrate.

94. The nucleic acid of claim 89, wherein the encoded polypeptide exhibits enantioselective lipase activity with respect to geranyl-butyrate.

20 95. The nucleic acid of claim 88, wherein the encoded polypeptide exhibits lipase activity with respect to methyl esters.

96. The nucleic acid of claim 88, wherein the encoded polypeptide exhibits lipase activity with respect to pentadecanolide.

97. The nucleic acid of claim 88, wherein the encoded polypeptide exhibits lipase activity with respect to oxacyclotridecan.

98. An isolated or recombinant nucleic acid comprising a polynucleotide sequence encoding a polypeptide comprising lipase activity produced by mutating or  
5 recombining one or more polynucleotide sequence of claim 81.

99. The nucleic acid of claim 98, wherein the encoded polypeptide comprises enantioselective lipase activity.

100. The nucleic acid of claim 81, 82, or 98, wherein the encoded polypeptide comprises a leader sequence.

101. The nucleic acid of claim 81, 82, or 98, wherein the encoded polypeptide  
10 comprises a precursor peptide.

102. The nucleic acid of claim 81, 82, or 98, wherein the encoded polypeptide comprises an epitope tag sequence.

103. The nucleic acid of claim 81, 82, or 98, wherein the nucleic acid encodes  
15 a fusion protein, said nucleic acid comprising one or more additional nucleic acid sequences.

104. A composition comprising two or more nucleic acids of claim 81, 82, or  
98.

105. A composition of claim 104, wherein the composition comprises a library comprising at least about 2, 5, 10, 50, or more of the nucleic acids.

106. A composition produced by cleaving of one or more nucleic acid of  
20 claim 81, 82, or 98.

107. The composition of claim 106, wherein the cleaving comprises mechanical, chemical or enzymatic cleavage.

108. The composition of claim 107, wherein the enzymatic cleavage comprises cleavage with a restriction endonuclease, an RNase or a DNase.

109. A composition produced by a process comprising incubating one or more nucleic acids of claim 81, 82, or 98, in the presence of deoxyribonucleotide triphosphates and  
5 a nucleic acid polymerase.

110. The composition of claim 109, wherein the nucleic acid polymerase is a thermostable polymerase.

111. A cell comprising at least one nucleic acid of claim 81, 82, or 98, or a cleaved or amplified fragment or product thereof.

112. The cell of claim 111, wherein the cell expresses a polypeptide encoded  
10 by the nucleic acid.

113. A vector comprising the nucleic acid of claim 81, 82, or 98.

114. The vector of claim 113, wherein the vector comprises a plasmid, a cosmid, a phage, a virus or a fragment of a virus.

115. The vector of claim 113, wherein the vector comprises an expression  
15 vector.

116. A cell transduced by the vector of claim 113.

117. A composition comprising a polypeptide encoded by a nucleic acid selected from the nucleic acids of claim 81, 82, or 98, and an excipient.

118. The composition of claim 117, wherein the excipient is a  
20 pharmaceutically acceptable excipient.

119. A composition comprising a polypeptide of claim 81, 82, or 98, wherein the composition comprises a cleaning solution.

120. The composition of claim 119, wherein the cleaning solution further comprises one or more of: a builder, a surfactant, a polymer, a bleach system, a structurant, a pH adjuster, a humectant, or a neutral inorganic salt.

121. A nucleic acid which comprises a unique subsequence in a nucleic acid  
5 selected from SEQ ID NO: 1 to SEQ ID NO: 54, wherein the unique subsequence is unique as compared to a nucleic acid sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406, BAA22231, BAB05967, C69652, CAA00273, CAA00274,  
10 CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

122. A target nucleic acid which hybridizes under stringent conditions to a  
15 unique coding oligonucleotide which encodes a unique subsequence in a polypeptide selected from SEQ ID NO: 55 to SEQ ID NO: 108, wherein the unique subsequence is unique as compared to an amino acid sequence or to a polypeptide encoded by a nucleic acid sequence corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217, AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406,  
20 BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621, CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903, E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and Z99108.

123. The nucleic acid of claim 122, wherein the stringent conditions are  
25 selected such that a perfectly complementary oligonucleotide to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least a 5x higher signal to noise ratio than for hybridization of the perfectly complementary oligonucleotide to a control nucleic acid corresponding to any of GenBank accession numbers: 1I6WA, 1I6WB, A02813, A02815, A34992, AAA22574, AAB31769, AAC12257, AAD30278, AAF40217,

AAF63229, AB000617, AF134840, AF141874, AF237623, AJ297356, BAA11406,  
BAA22231, BAB05967, C69652, CAA00273, CAA00274, CAA02196, CAA64621;  
CAB12064, CAB12664, CAB51971, CAB92662, CAB95850, D78508, E01340, E01903,  
E02083, E05047, JW0068, M74010, P37957, S23934, U78785, X95309, Z99105, and  
5 Z99108, wherein the target nucleic acid hybridizes to the unique coding oligonucleotide with  
at least about a 2x higher signal to noise ratio as compared to hybridization of the control  
nucleic acid to the coding oligonucleotide.

124. A database comprising one or more character strings corresponding to a  
polynucleotide sequence selected from SEQ ID NO: 1 to SEQ ID NO: 54 or a polypeptide  
10 sequence selected from SEQ ID NO: 55 to SEQ ID NO: 108.

125. The database of claim 124, wherein the one or more character strings is  
recorded in a computer readable medium.

126. The database of claim 125, wherein the computer readable medium  
comprises a medium that resides internal or external to a computer.

15 127. A method for manipulating a sequence record in a computer system, the  
method comprising:

(a) reading a character string corresponding to a polynucleotide sequence  
selected from SEQ ID NO: 1 to SEQ ID NO: 54 or a polypeptide sequence selected from  
SEQ ID NO: 55 to SEQ ID NO: 108 or a subsequence thereof;

20 (b) performing an operation on the character string; and,

(c) returning a result of the operation.

128. The method of claim 127, comprising reading a character string selected  
by a user.

25 129. The method of claim 128, wherein the user selects the character string  
from a database or inputs the character string into the computer system.

130. The method of claim 127, comprising transmitting the selected character string to an output device.

131. The method of claim 127, comprising performing one or more operations selected from among: a local sequence comparison, a sequence alignment, a sequence  
5 identity or similarity search, a structural similarity search, a sequence identity or similarity determination, a structure determination, a nucleic acid motif determination, an amino acid motif determination, a hypothetical translation, a determination of a restriction map, a sequence recombination, or a BLAST determination.

132. The method of claim 131, comprising aligning the selected character  
10 string with one or more additional character strings corresponding to a polynucleotide or polypeptide sequence.

133. The method of claim 131, comprising translating one or more character strings selected from SEQ ID NO: 1 to SEQ ID NO: 54, into a character string corresponding to an amino acid sequence or translating a character string selected from SEQ ID NO: 55 to  
15 SEQ ID NO: 108, into a character string corresponding to a polynucleotide sequence.

134. The method of claim 131, comprising determining sequence identity or similarity between the selected character string and one or more additional character strings, by evaluating codon usage.

135. The method of claim 134, comprising determining optimal codon usage.

20 136. The method of claim 127, comprising obtaining the result of the operation on a user output device.

137. The method of claim 136, wherein the user output device is selected from among: a display monitor, a printer, and an audio-output.

25 138. The method of claim 127, wherein the operation comprises transmitting the character string to a device capable of producing a physical embodiment of the character string.



139. The method of claim 138, comprising obtaining the result by obtaining a nucleic acid corresponding to the character string.

140. The method of claim 138, comprising obtaining the result by obtaining a polypeptide or peptide corresponding to the character string or a sub-portion thereof.

5 141. The method of claim 138, wherein the device comprises an oligonucleotide synthesizer.

142. The method of claim 138, wherein the device comprises a peptide synthesizer.

10 143. A method of producing a modified or recombinant nucleic acid comprising mutating or recombining a nucleic acid of claim 81 or 82.

144. A modified or recombinant nucleic acid produced by the method of claim 143.

145. The method of claim 143, comprising recursively recombining the nucleic acid with one or more additional nucleic acids.

15 146. A modified or recombinant nucleic acid produced by the method of claim 145.

147. The method of claim 145, wherein the one or more additional nucleic acids encode a polypeptide comprising lipase activity or an amino acid subsequence or fragment thereof.

20 148. The method of claim 145, wherein the recursive recombination is performed in vitro.

149. The method of claim 145, wherein the recursive recombination is performed in vivo.

150. The method of claim 145, wherein the recursive recombination produces at least one library of recombinant nucleic acids, which library comprises at least one polypeptide comprising lipase activity, or a homologue thereof.

151. A nucleic acid library produced by the method of claim 150.

5 152. A population of cells comprising the library of claim 151.

153. The modified or recombinant nucleic acid produced by the method of claim 143.

154. A cell comprising the nucleic acid of claim 153.

10 155. A method of producing a polypeptide, the method comprising: (a) introducing a nucleic acid of claim 81, 82, or 98, into a population of cells, which nucleic acid is operably linked to a regulatory sequence capable of directing expression of a polypeptide encoded by the nucleic acid in at least a subset of the population of cells or progeny thereof; and, (b) expressing the polypeptide in at least the subset of the population of cells or progeny thereof.

15 156. The method of claim 155, further comprising isolating the polypeptide from the cells.

157. The method of claim 155, comprising expressing the polypeptide by culturing the population or subset of the population of cells in a nutrient medium under conditions in which the regulatory sequence directs expression of the polypeptide encoded by the nucleic acid.

20

158. The method of claim 155, further comprising isolating or recovering the polypeptide from the cells or from the nutrient medium.

159. The method of claim 155, wherein the culturing is performed in a bulk fermentation vessel.

**160.** The method of claim 155, wherein the cells are bacterial cells.

**161.** The method of claim 155, wherein the cells are eukaryotic cells.

**162.** The method of claim 161, wherein the cells are fungal cells, yeast cells, plant cells, insect cells, or mammalian cells.

5           **163.** The method of claim 162, wherein the mammalian cells comprise fertilized oocytes, embryonic stem cells, or pluripotent stem cells, further comprising regenerating a transgenic mammal expressing the polypeptide, and recovering the polypeptide from the transgenic mammal or a by-product of the transgenic animal.

**164.** The method of claim 163, wherein the by-product is milk.

10           **165.** A polypeptide produced by the method of claim 155.

**166.** A method of hydrolyzing a lipid to therapeutically or prophylactically treat a gastrointestinal lipid related condition/disease/disorder, the method comprising: expressing in a target cell or contacting a target cell with an effective amount of a polypeptide of claim 1 or 47.

15

Clone name	SEQ ID NO	Enantioselectivity for Neryl Butyrate (N) or Geranyl Butyrate (G)
1F15 (G2)	21	G
3C12	22	G
3N19 (G2)	23	G
G2.2	24	G
2C3	25	G
2F11	26	G
KV11 (6C7)	27	N
KV6 (3A1)	28	N
KV2 (2D1)	29	N
N2.5	30	N
KV5 (2H6)	31	N
3E5	32	G
G2.1	33	G
3H24 (G2)	34	G
KV10 (4G6)	35	N
KV12 (6D4)	36	N
N2.2	37	N
N2.3	38	N
N2.1	39	N
KV4 (2E12)	40	N
KV9 (4C6)	41	N
7D6	42	G
3F3	43	G
2D11 (G2)	44	G
3C23 (G2)	45	G
G2.3	46	G
2A3	47	G
2F4	48	G
2B9 (G2)	49	G
2C5	50	G
KV1 (2A6)	51	N
2D13 (G2)	52	G
3C8	53	G
2D5	54	G

FIGURE 1

Clone Name	E value for Neryl Butyrate	E value for Geranyl Butyrate
Exemplar (sgc2 and sgd2)	---	2.1
Exemplar (2h6)	1.4	---
Exemplar (14g14)	1.8	---
Exemplar (3f19a11)	2.2	(not tested)
Exemplar (3e5)	---	3.0
Exemplar (3n19)	---	3.8

FIGURE 2

SEQ:001-405 (pumilus) (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:002-406 (subtilis) (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCCTGTACATCGC  
 SEQ:003-402 (megat.) (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:004-400 (lentus) (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:005-396 (circul.) (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:006-392 (azotof.) (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:007-398 (flrmus) (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:008-393 (badius) (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:009-Dc5h (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:010-Dc5f (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:011-Dc5c1 (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:012-Dc5a2 (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:013-Dc5l2 (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:014-Sga (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:015-Sgc (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:016-Sgd (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:017-Sgf (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:018-Sgh (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:019-Mt2b1 (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC  
 SEQ:020-H2a (1) -----ATGAAATTTGTA AAAAAGGATCAATTGCA ----CTTGTAAACAATTTTGGTGTCTG-TCAGTCACATCGC

75

Figure 3a

	(Signal peptide coding region)	(Mature coding region)
SEQ: 001-405 (pumlilus)	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 002-406 (subtilisin)	(65) TGTITGGGTTGCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTCGTTATGGTTTCAC-GGTATTTGGAGGGGCA
SEQ: 003-402 (megat.)	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GA-CACAATCCAGTTGTTATGGTTTCACCTGGTATCGGAGGAGCT
SEQ: 004-400 (lentus)	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 005-396 (circul.)	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 006-392 (azotof.)	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 007-398 (firmus)	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 008-393 (badius)	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 009-Dc5h	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 010-Dc5f	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 011-Dc5c1	(75) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 012-Dc5a2	(75) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 013-Dc512	(75) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 014-Sga	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 015-Sgc	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 016-Sgd	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 017-Sgf	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 018-Sgh	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 019-Mt2b1	(75) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT
SEQ: 020-H2a	(65) TGTITGGGATGTCAGCCGTCAGCAAAAAGCCGCT	GAACACAATCCAGTTGTTATGGTTTCAC-GGTATCGGAGGAGCT

Figure 3b

225

151

SEQ:001-405 (pum11us) (139) TCATACAATTTGCGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
 SEQ:002-406 (sub111is) (139) TCATTCATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGAGTTGAT  
 SEQ:003-402 (megat.) (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
 SEQ:004-400 (lentus) (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
 SEQ:005-396 (circul.) (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
 SEQ:006-392 (azotof.) (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
 SEQ:007-398 (firmus) (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
 SEQ:008-393 (badius) (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:009-Dc5h (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:010-Dc5f (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:011-Dc5c1 (148) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:012-Dc5a2 (148) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:013-Dc512 (148) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:014-Sga (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:015-Sgc (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:016-Sgd (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:017-Sgf (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:018-Sgh (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:019-Mt2b1 (148) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT  
     SEQ:020-H2a (139) TCATACAATTTTTCGGGGAAATTAAGAGCTATCTCGTATCTCAGGCTGGTACGGGGCAAGCTGTATGCGGGTTGAT

Figure 3c



226  
 SEQ:001-405 (pumillus) (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:002-406 (subtilis) (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:003-402 (megat.) (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:004-400 (lentus) (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:005-396 (circul.) (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:006-392 (azotof.) (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:007-398 (firmus) (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:008-393 (badius) (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:009-Dc5h (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:010-Dc5f (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:011-Dc5c1 (223) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:012-Dc5a2 (223) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:013-Dc512 (223) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:014-Sga (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:015-Sgc (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:016-Sgd (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:017-Sgf (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:018-Sgh (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:019-Mt2b1 (223) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA  
 SEQ:020-H2a (214) TTTTGGGACAAGACAGGACGGAATTATAACAATGGCCCGGTATTATCACGATTTGTGCAAAAGGTTTTAGACGAA

226

300

Figure 3d

301. SEQ: 001-405 (pumilus) (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGTATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 002-406 (subtilis) (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 003-402 (megat.) (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 004-400 (lentus) (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 005-396 (circul.) (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 006-392 (azotof.) (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 007-398 (firmus) (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 008-393 (badius) (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 009-Dc5h (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 010-Dc5f (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGGTGGCCGGAACACACCTTACTACATAAAAAATCTG  
 SEQ: 011-Dc5c1 (298) ACGGGCCGAAAAAAGTGGATATTGTCGCTCATAGTATGGCGGTGCGGAACACCGTTTACTATATTAAGAACTA  
 SEQ: 012-Dc5a2. (298) ACAGGTCCGAAAAAAGTGGATATTGTCGCTCATAGTATGGCGGTGCGGAACACCGTTTACTATATTAAGAACTA  
 SEQ: 013-Dc512 (298) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCATAGTATGGGTGGAGGAAACACCGTTTACTATATTAAGAACTA  
 SEQ: 014-Sga (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGCGGTGCGGAACACCGTTTACTACATAAAAAATTTG  
 SEQ: 015-Sgc (289) ACCGGTCCGAAAAAAGTGGATATTGTCGCTCACAGCATGGCGGTGCGGAACACCGTTTACTACATAAAAAATTTG  
 SEQ: 016-Sgd (289) ACAGGAGCCAAAAAAGTGGATATTGTCGCTCATAGTATGGCGGTGCGGAACACCATTTACTATATTAAGAACTA  
 SEQ: 017-Sgf (289) ACAGGAGCCAAAAAAGTGGATATTGTCGCTCATAGTATGGCGGTGCGGAACACCATTTACTATATTAAGAACTA  
 SEQ: 018-Sgh (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCATAGTATGGCGGTGCGGAACACCGTTTACTATATTAAGAACTA  
 SEQ: 019-Mt2b1 (298) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCATAGTATGGCGGTGCGGAACACCGTTTACTATATTAAGAACTA  
 SEQ: 020-H2a (289) ACGGGTCCGAAAAAAGTGGATATTGTCGCTCATAGTATGGCGGTGCGGAACACCGTTTACTATATTAAGAACTA

Figure 3e

376  
 SEQ: 001-405 (pumilus) (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 002-406 (subtilis) (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 003-402 (megat.) (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 004-400 (lentus) (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 005-396 (circul.) (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 006-392 (azotof.) (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 007-398 (firmus) (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 008-393 (badius) (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 009-Dc5h (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 010-Dc5f (364) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 011-Dc5c1 (373) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 012-Dc5a2 (373) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 013-Dc5l2 (373) GACGGCGGAAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 014-Sga (364) GATGGCGCGGATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 015-Sgc (364) GATGGCGGTAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 016-Sgd (364) GATGGCGGTAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 017-Sgf (364) GATGGCGGTAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 018-Sgh (364) GATGGCGGTAATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 019-Mt2b1 (373) GATGGCGGCGGATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG  
 SEQ: 020-H2a (364) GATGGCGGCGGATAAAATTGAAAACGTCGTAACGCTTGGCGGCGGAACCCCTTCGACGACCAAGCAAGGCGCTTCCG

450

Figure 3f

451  
 SEQ:001-405 (pumilus) (439) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:002-406 (subtilis) (439) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:003-402 (megat.) (439) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:004-400 (lentus) (439) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:005-396 (circul.) (439) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:006-392 (azotof.) (439) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:007-398 (firmus) (439) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:008-393 (badlus) (439) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:009-Dc5h  
 SEQ:010-Dc5f (439) GGCACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:011-Dc5c1 (448) GGCACCGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:012-Dc5a2 (448) GGCACCGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:013-Dc5l2 (448) GGCACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:014-Sga (439) GGTACTGATCCCAACCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:015-Sgc (439) GGTACTGATCCCAACCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:016-Sgd (439) GGTACTGATCCCAACCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:017-Sgf (439) GGCACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:018-Sgh (439) GGCACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:019-Mt2b1 (448) GGAACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT  
 SEQ:020-H2a (439) GGCACAGATCCAAATCAAAGATTTTATACACATCCATTACA-GCAGTGCCGATATGATTTGTCATGAATTACTTT

525

Figure 39

SEQ: 001-405 (pumilus) (513) ATCAAAATTAGACGGT-GCTAAAAAC-GCTCAAAATTCATGGCGTTGGGCACATTTGGTTTATTGATGAACAGCCAA  
 SEQ: 002-406 (subtilis) (513) ATCAAGATTAGATGT-GCTAGAAC-GTTCAAATCCATGGCGTTGGACACATCGGCCCTTCTGTACAGCAGCCAA  
 SEQ: 003-402 (megat.) (513) ATCAAAATTAGACGGT-GCTAAAAAC-GTTCAAATTCATGGCGTTGGGCACATTTGGTTTATTGATGAACAGCCAA  
 SEQ: 004-400 (lentus) (513) ATCAAAATTAGACGGT-GCTAAAAAC-GTTCAAATTCATGGCGTTGGGCACATTTGGTTTATTGATGAACAGCCAA  
 SEQ: 005-396 (circul.) (513) ATCAAAATTAGACGGT-GCTAAAAAC-GTTCAAATTCATGGCGTTGGGCACATTTGGTTTATTGATGAACAGCCAA  
 SEQ: 006-392 (azotof.) (513) ATCAAAATTAGACGGT-GCTAAAAAC-GTACAAAATTCATGGCGTTGGGCACATTTGGTTTATTGATGAACAGCCAA  
 SEQ: 007-398 (firmus) (513) ATCAAAATTAGACGGT-GCTAAAAAC-GTCAAAATTCATGGCGTTGGGCACATTTGGTTTATTGATGAACAGCCAA  
 SEQ: 008-393 (badius) (513) ATCAAAATTAGACGGT-GCTAAAAAC-GTTCAAATTCATGGCGTTGGGCACATTTGGTTTATTGATGAACAGCCAA  
 SEQ: 009-De5h  
 SEQ: 010-De5f (513) ATCAAGATTAGATGT-GCGAGAAC-GTTCAAATCCATGGCGTTGGACACATCGGCCCTTCTGTACAGCAGCCAA  
 SEQ: 011-De5c1 (522) CTCGCTTAATTGCG-GCAAGAAC-ATCCTGATCCATGGCGTTGGACATATCGGCTATTAACCTCAAGCCAA  
 SEQ: 012-De5a2 (522) TTCGC-GTTTTAATTGGCGCAAGAAC-GTCCTGATCCACGGCGTTGGACATATCGGCTATTAACCTCAAGCCAA  
 SEQ: 013-De512 (522) CTCAGTTTTAATTGGCGCAAGAAC-ATCCTGATCCA-GGCGTTGGTCATATCGGCTATTAACCTCAAGCCAA  
 SEQ: 014-Sga (513) AACAAAATTAGACGGG-GCTAAAAAT-GTTCAAATTCATGGGTGTCGGACATATCGGCCCTTCTGTACAGCAGCCAA  
 SEQ: 015-Sgc (513) ATCAAAATTAGACGGG-GCTAAAAAT-GTTCAAATTCATGGGTGTCGGACATATCGGCCCTTCTGTACAGCAGCCAA  
 SEQ: 016-Sgd (513) ATCAAAATTAGACGGG-GCTAAAAAT-GTTCAAATTCATGGGTGTCGGACATATCGGCCCTTCTGTACAGCAGCCAA  
 SEQ: 017-Sgf (513) CTCGCTTAATTGCG-GCAAGAAC-GTCCAAAATCCATGGCGTTGGACATATCGGCTATTAACCTCAAGCCAA  
 SEQ: 018-Sgh (513) CTCGCTTAATTGCG-GCAAGAAC-GTCCAAAATCCATGGCGTTGGACATATCGGCTATTAACCTCAAGCCAA  
 SEQ: 019-Mc2b1 (522) TTCGC-GTTTTAATTGGCGCAAGAAC-GTCCTGATCCACGGCGTTGGCCATATCGGCTATTAACCTCAAGCCAA  
 SEQ: 020-H2a (514) CTCGCTTAATTGGCTGCAAGAACAGTCCAAAATCCATGGCGTTGGACATATCGGCTATTAACCTCAAGCCAA

600

526

Figure 3h

601  
 SEQ: 001-405 (pumilus) (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCCAAAATACGAATTAA  
 SEQ: 002-406 (subtilis) (586) GTCAACAGCCTGATTTAAAGAAGGCTGAACGGCGGGGACTCAATACAAATTAG  
 SEQ: 003-402 (megat.) (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCCACAATACAAATTAA  
 SEQ: 004-400 (lentus) (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGACTAAATACAAATTAA  
 SEQ: 005-396 (circul.) (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAANTACAAATTAA  
 SEQ: 006-392 (azotof.) (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTAGATACAAATTAA  
 SEQ: 007-398 (firmus) (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGACCAATACAAATTAA  
 SEQ: 008-393 (badius) (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGACCAATACAAATTAA  
 SEQ: 009-Dc5h (586) GTCAACAGCCTGATTTAAAGAAGGCTGAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 010-Dc5f (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 011-Dc5c1 (595) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 012-Dc5a2 (595) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 013-Dc512 (595) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 014-Sga (586) GTCAACAGCCTGATTTAAAGAAGGCTTAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 015-Sgc (586) GTCAACAGCCTGATTTAAAGAAGGCTTAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 016-Sgd (586) GTCAACAGCCTGATTTAAAGAAGGCTTAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 017-Sgf (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 018-Sgh (586) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 019-Mc2b1 (595) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAATACAAATTAA  
 SEQ: 020-H2a (589) GTCAACAGCCTGATTTAAAGAAGGACTGAACGGCGGGGCTCAATACAAATTAA

654

Figure 3i

75

1. SEQ: 021-1f15 (G2) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 022-3C12 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 023-3N19 (G2) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 024-G2.2 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 025-2C3 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 026-2F11 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 027-KV11 (6C7) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 028-KV5 (3A1) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 029-KV2 (2D1) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 030-N2.5 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 031-KV5 (2H6) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 032-3E5 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 033-G2.1 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 034-3H24 (G2) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 035-KV10 (4G6) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 036-KV12 (6D4) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 037-N2.2 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 038-N2.3 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 039-N2.1 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 040-KV4 (2E12) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 041-KV9 (4C6) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 042-7D6 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 043-3F3 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 044-2D11 (G2) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 045-3C23 (G2) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 046-G2.3 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 047-2A3 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 048-2F4 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 049-2B9 (G2) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 050-2C5 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 051-KV1 (2A6) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 052-2D13 (G2) (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 053-3C8 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

SEQ: 054-2D5 (1) TGAACACAATCCAGTTGTTATGGTTACGGTATTGGAGGGGCATCAATCAATTTTCGGGGAAATTAAGAGCTATCT

Figure 4a

SEQ: 021-1f15 (G2) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 022-3C12 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 023-3N19 (G2) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 024-G2.2 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 025-2C3 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 026-2F11 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 027-KV11 (6C7) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 028-KV6 (3A1) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 029-KV2 (2D1) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 030-N2.5 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 031-KV5 (2H6) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 032-3E5 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 033-G2.1 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 034-3H24 (G2) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 035-KV10 (4G6) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 036-KV12 (6D4) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 037-N2.2 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 038-N2.3 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 039-N2.1 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 040-KV4 (2E12) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 041-KV9 (4C6) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 042-7D6 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 043-3F3 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 044-2D11 (G2) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 045-3C23 (G2) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 046-G2.3 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 047-2A3 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 048-2F4 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 049-2B9 (G2) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 050-2C5 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 051-KV1 (2A6) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 052-2D13 (G2) (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 053-3C8 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA  
 SEQ: 054-2D5 (76) CGTATCTCAGGGCTGGTCCGGGGCAAGCTGTATGCCGTTGATTTTGGGACAAGACAGGGACGAAATTATAACAA

Figure 4b



151 225

SEQ: 021-1f15 (G2) (151) TGGCCCGGTATTATCGCGTTTGTGAAAAAGGTATTAGATAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 022-3C12 (151) TGGCCCGGTATTATAGATTCGTCAAAAGTTCGTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 023-3N19 (G2) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 024-G2.2 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 025-2C3 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 026-2F11 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 027-KV11 (6C7) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 028-KV6 (3A1) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 029-KV2 (2D1) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 031-KV5 (2H6) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 032-3E5 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 033-G2.1 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 034-3H24 (G2) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 035-KV10 (4G6) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 036-KV12 (6D4) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 037-N2.2 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 038-N2.3 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 039-N2.1 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 040-KV4 (2E12) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 041-KV9 (4C6) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 042-7D6 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 043-3F3 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 044-2D11 (G2) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 045-3C23 (G2) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 046-G2.3 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 047-2A3 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 048-2F4 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 049-2B9 (G2) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 050-2C5 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 051-KV1 (2A6) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 052-2D13 (G2) (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 053-3C8 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA  
 SEQ: 054-2D5 (151) TGGCCCGGTATTATCAGATTTGTGCAAAAGGTATTAGACAAACGGGTGCGAAAAAAGTGGATATTGTCGGCTCA

Figure 4 c

300

226  
 SEQ: 021-1f15 (G2) (226) CAGCATGGGCGGGCTTAACACACGCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAAGTTGAAAAACGTCGTAAC  
 SEQ: 022-3C12 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 023-3N19 (G2) (226) CAGCATGGGTGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 024-G2.2 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 025-2C3 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 026-2F11 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 027-KV11 (6C7) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 028-KV6 (3A1) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 029-KV2 (2D1) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 030-N2.5 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 031-KV5 (2H6) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 032-3E5 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 033-G2.1 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 034-3H24 (G2) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 035-KV10 (4G6) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 036-KV12 (6D4) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 037-N2.2 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 038-N2.3 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 039-N2.1 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 040-KV4 (2E12) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 041-KV9 (4C6) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 042-7D6 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 043-3F3 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 044-2D11 (G2) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 045-3C23 (G2) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 046-G2.3 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 047-2A3 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 048-2F4 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 049-2B9 (G2) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 050-2C5 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 051-KV1 (2A6) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 052-2D13 (G2) (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 053-3C8 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC  
 SEQ: 054-2D5 (226) CAGCATGGGCGGGCGGAAACACACCTTTACTACATATAAAAAATCTGGACGGCGGAAATAAATAATTGAAAAACGTCGTAAC

Figure 4d

375

301

SEQ: 021-1F15 (G2) (301) GCTTGGCGGCACGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 022-3C12 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 023-3N19 (G2) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 024-G2.2 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 025-2C3 (301) CATTGGTGGAGCAAAACCGACTCGTTTCAAGCAGACATACCAGGCACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 026-2F11 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 027-KV11 (6C7) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 028-KV6 (3A1) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 029-KV2 (2D1) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 030-N2.5 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 031-KV5 (2H6) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 032-3E5 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 033-G2.1 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 034-3H24 (G2) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 035-KV10 (4G6) (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 036-KV12 (6D4) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 037-N2.2 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 038-N2.3 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 039-N2.1 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 040-KV4 (2E12) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 041-KV9 (4C6) (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 042-7D6 (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 043-3F3 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 044-2D11 (G2) (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 045-3C23 (G2) (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 046-G2.3 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 047-2A3 (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 048-2F4 (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 049-2B9 (G2) (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 050-2C5 (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 051-KV1 (2A6) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 052-2D13 (G2) (301) GCTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 053-3C8 (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC  
 SEQ: 054-2D5 (301) ACTTGGCGGCAGAACCGTTTCGACGACAAGCAAGGCGCTTCCGGGAACAGATCCAAATCAAAAGATTTTATACAC

Figure 4e

376 SEQ:021-1f15(G2) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTAATTTACAAAATTTAGACGGTGTAAAAATGTTCAAAT  
SEQ:022-3C12 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTAATTTACAAAATTTAGACGGGCTAAAAATGTTCAAAT  
SEQ:023-3N19(G2) (376) ATCCATTTACGGCAGTCCCGATATGATTTGTCATCAAAATTAATTTACAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:024-G2.2 (376) ATCCATTTACGGCAGTCCCGATATGATTTGTCATCAAAATTAATTTACAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:025-2C3 (376) ATCCGTTTACAGTCCAGCAGATCTTATTTGTCGTCACACAGTCTCTCTCGTTTAAATTTGGCGCAAGAAAACGTTCAAAT  
SEQ:026-2F1.1 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:027-KV11(6C7) (376) ATCCGTTTACAGTCCCGATATGATTTGTCATCAAAATTTAGACGGGCTAAAAATGTTCAAAT  
SEQ:028-KV6(3A1) (376) ATCCGTTTACAGTCCCGATATGATTTGTCATCAAAATTTAGACGGGCTAAAAATGTTCAAAT  
SEQ:029-KV2(2D1) (376) ATCCGTTTACAGTCCCGATATGATTTGTCATCAAAATTTAGACGGGCTAAAAATGTTCAAAT  
SEQ:030-N2.5 (376) ATCCGTTTACAGTCCCGATATGATTTGTCATCAAAATTTAGACGGGCTAAAAATGTTCAAAT  
SEQ:031-KV5(2H6) (376) ATCCGTTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:032-3E5 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGGCTAAAAATGTTCAAAT  
SEQ:033-G2.1 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:034-3H24(G2) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:035-KV10(4G6) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:036-KV12(5D4) (376) ATCCGTTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:037-N2.2 (376) ATCCGTTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:038-N2.3 (376) ATCCGTTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:039-N2.1 (376) ATCCGTTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:040-KV4(2E12) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:041-KV9(4C6) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:042-7D6 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:043-3F3 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:044-2D11(G2) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:045-3C23(G2) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:046-G2.3 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:047-2A3 (376) ATCCGTTTACAGTCCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:048-2F4 (376) ATCCGTTTACAGTCCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:049-2B9(G2) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGGCTAAAAATGTTCAAAT  
SEQ:050-2C5 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:051-KV1(2A6) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:052-2D13(G2) (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:053-3C8 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAACGTTCAAAT  
SEQ:054-2D5 (376) ATCCATTTACAGCAGTCCCGATATGATTTGTCATCAAAATTTAGACGGTGTAAAAATGTTCAAAT

450

376

Figure 4f

451  
 SEQ: 021-1f15 (G2) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 022-3C12 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 023-3N19 (G2) (451) CCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 024-G2.2 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 025-2C3 (451) CCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 026-2F11 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 027-KV11 (6C7) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 028-KV6 (3A1) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 029-KV2 (2D1) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 030-N2.5 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 031-KV5 (2H6) (451) CCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 032-3E5 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 033-G2.1 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 034-3H24 (G2) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 035-KV10 (4G6) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 036-KV12 (6D4) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 037-N2.2 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 038-N2.3 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 039-N2.1 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 040-KV4 (2E12) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 041-KV9 (4C6) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 042-7D6 (451) CCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 043-3F3 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 044-2D11 (G2) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 045-3C23 (G2) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 046-G2.3 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 047-2A3 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 048-2F4 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 049-2B9 (G2) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 050-2C5 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 051-KV1 (2A6) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 052-2D13 (G2) (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 053-3C8 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG  
 SEQ: 054-2D5 (451) TCATGGCGTTGGGCACATGGTTTATTGATGAACAGCCAAAGTCAACAGCCTGATTAAGAAGGACTGAACGGCGG

Figure 4g

	526	544
SEQ: 021-1f15 (G2)	(526)	GGGACTCAATACGGAATTGA
SEQ: 022-3C12	(526)	GGGACTCAATACGGAATTGA
SEQ: 023-3N19 (G2)	(526)	GGGACTGAATACAAATTGA
SEQ: 024-G2.2	(526)	GGGACTCAATACGGAATTGA
SEQ: 025-2C3	(526)	GGCCACAATACGGAATTGA
SEQ: 026-2F11	(526)	AGGCTAAATACGGAATTGA
SEQ: 027-KV11 (6C7)	(526)	GGGCTAAATACAAATTGA
SEQ: 028-KV6 (3A1)	(526)	GGGCTAAATACAAATTGA
SEQ: 029-KV2 (2D1)	(526)	GGCCAAAATACAAATTGA
SEQ: 030-N2.5	(526)	GGCCACAATACAAATTGA
SEQ: 031-KV5 (2H6)	(526)	GGGCTGAATACAAATTGA
SEQ: 032-3E5	(526)	GGGCTCAATACGGAATTGA
SEQ: 033-G2.1	(526)	GGGACTCAATACGGAATTGA
SEQ: 034-3H24 (G2)	(526)	GGGACTCAATACGGAATTGA
SEQ: 035-KV10 (4G6)	(526)	GGCCACAATACAAATTGA
SEQ: 036-KV12 (6D4)	(526)	AGCCACAATACAAATTGA
SEQ: 037-N2.2	(526)	AGCCACAATACAAATTGA
SEQ: 038-N2.3	(526)	AGCCACAATACAAATTGA
SEQ: 039-N2.1	(526)	AGCCACAATACAAATTGA
SEQ: 040-KV4 (2E12)	(526)	GGCCACAATACGGAATTGA
SEQ: 041-KV9 (4C6)	(526)	GGCCACAATACGGAATTGA
SEQ: 042-7D6	(526)	GGGATTAATACGGAATTGA
SEQ: 043-3F3	(526)	GGCCAGAATACGGAATTGA
SEQ: 044-2D11 (G2)	(526)	AGCCAGAATACGGAATTGA
SEQ: 045-3C23 (G2)	(526)	GGCCACAATACGGAATTGA
SEQ: 046-G2.3	(526)	GGCCAGAATACGGAATTGA
SEQ: 047-2A3	(526)	AGGCTAAATACAAATTGA
SEQ: 048-2F4	(526)	AGCCAGAATACGGAATTGA
SEQ: 049-2B9 (G2)	(526)	AGCCAAAATACGGAATTGA
SEQ: 050-2C5	(526)	AGCCAAAATACGGAATTGA
SEQ: 051-KV1 (2A6)	(526)	GGCCAGAATACGGAATTGA
SEQ: 052-2D13 (G2)	(526)	AGCCAAAATACGGAATTGA
SEQ: 053-3C8	(526)	GGCCAAAATACAAATTGA
SEQ: 054-2D5	(526)	AGGACAAAATACAAATTGA

Figure 4 h

	(Signal peptide)	(Mature region)
SEQ: 055-405 (pumilus)	(1) --MKFVKRRRIIALVTLILVLSVTSILFAMQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRGKLYAVDF
SEQ: 056-406 (subtilis)	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 057-402 (megat.)	(1) --MKFVKRRRIIALVTLILVLSVTSILFAMQP-SAKAA	1 DTIQLLWFTIGGGASYNFAGIKSYLVSQGWSRGKLYAVDF
SEQ: 058-400 (lentus)	(1) --MKFVKRRRIIALVTLILVLSVTSILFAMQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRGKLYAVDF
SEQ: 059-396 (circul.)	(1) --MKFIKRRRIIALVTLILVLSVTSILFAMQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRGKLYAVDF
SEQ: 060-392 (azotof.)	(1) --MKFVKRRRIIALVTLILVLSVTSILFAMQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRGKLYAVDF
SEQ: 061-398 (firmus)	(1) --MKFVKRRRIIALVTLILVLSVTSILFAMQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRGKLYAVDF
SEQ: 062-393 (badius)	(1) --MKFVKRRRIIALVTLILVLSVTSILFAMQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRGKLYAVDF
SEQ: 063-Dc5h	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 064-Dc5f	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 065-Dc5c1	(1) MKVIFVKRRSLQILVALALVLSGSAFIQPKEAKAA	1 EHNPVVMVHGMGGASYNFASIKRYLVSQGWDRNQLFAIDF
SEQ: 066-Dc5a2	(1) MKVIFVKRRSLQILVALALVIGMAFIQPKEAKAA	1 EHNPVVMVHGIIGGASYNFFSIKSYLATQGWDRNQLYAIIDF
SEQ: 067-Dc512	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 068-Sga	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 069-Sgc	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 070-Sgd	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 071-Sgf	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 072-Sgh	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF
SEQ: 073-Mt2b1	(1) MKVIFVKRRSLQILVALALVIGMAFIQPKEIKAA	1 EHNPVVMVHGIIGGASYNFASIKSYLVNQGWSRDKLYAIIDF
SEQ: 074-H2a	(1) --MKFVKRRRIIALVTLILVLSVTSILFALQP-SAKAA	1 EHNPVVMVHGIIGGASYNFAGIKSYLVSQGWSRDKLYAVDF

Figure 5a

115

41

SEQ: 055-405 (pumilus) (73) WDKTGTNNNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPG  
 SEQ: 056-406 (subtilis) (73) WDKTGTNNNGPVL PRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKVANVVTLGGANRLTTGKALPG  
 SEQ: 057-402 (megat.) (73) WDKTGTNNNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPG  
 SEQ: 058-400 (lentus) (73) WDKTGTNNNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPG  
 SEQ: 059-396 (circul.) (73) WDKTGTNNNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPG  
 SEQ: 060-392 (azotof.) (73) WDKTGTNNNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPG  
 SEQ: 061-398 (firmus) (73) WDKTGTNNNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPG  
 SEQ: 062-393 (badius) (73) WDKTGTNNNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPG  
 SEQ: 063-Dc5h (73) XDKTGNRRNGPRL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGDKIENVVTLGGANGLVSSRALPG  
 SEQ: 064-Dc5f (73) KDKTGTNNNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKVENVVTLGGANRLTTGKALPG  
 SEQ: 065-Dc5c1 (76) IDKTGNLNLNGPRL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGDKIENVVTLGGANGLVSLRALPG  
 SEQ: 066-Dc5a2 (76) IDKTGNRRNGPRL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGDKIENVVTLGGANGLVSLRALPG  
 SEQ: 067-Dc512 (76) IDKTGNRRNGPRL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGDKIENVVTLGGANGLVSSRALPG  
 SEQ: 068-Sga (73) RDKTGNLNLNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLVTGKALPG  
 SEQ: 069-Sgc (73) WDKTGNLNLNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLVTGKALPG  
 SEQ: 070-Sgd (73) SDKTGNLNLNGPVL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGNKIENVVTLGGANRLVTGKALPG  
 SEQ: 071-Sgf (73) KDKTGNRRNGPRL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGDKIENVVTLGGANGLVSSRALPG  
 SEQ: 072-Sgh (73) IDKTGNRRNGPRL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGDKIENVVTLGGANGLVSSRALPG  
 SEQ: 073-Wt2b1 (76) IDKTGNRRNGPRL SRFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGDKIENVVTLGGANGLVSLRALPG  
 SEQ: 074-H2a (73) RDKTGNRRNGPRL SKFVQKVLDETGAKKVD IVAHSMGGANTLYYIKNLDGGDKIENVVTLGGANGLVSSRALPG

Figure 5b



116 (148) TDPNQKILYTSIYSSADMIVMN-YLSKLDGAKNAQIHGVGHI GLLMNSQVNSLIKEGLNGGGQNTN  
 SEQ: 055-405 (pumilus)  
 (148) TDPNQKILYTSIYSSADMIVN-YLSRLDGARNVQIHGVGHI GLLYSSQVNSLIKEGLNGGGLNTN  
 SEQ: 056-406 (subtilis)  
 (148) TDPNQKILYTSIYSSADMIVMN-YLSKLDGAKNVQIHGVGHI GLLMNSQVNSLIKEGLNGGGHNTN  
 SEQ: 057-402 (megat.)  
 (148) TDPNQKILYTSIYSSADMIVMN-YLSKLDGAKNVQIHGVGHI GLLMNSQVNSLIKEGLNGGGHNTN  
 SEQ: 058-400 (lentus)  
 (148) TDPNQKILYTSIYSSADMIVMN-YLSKLDGAKNVQIHGVGHI GLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 059-396 (circul.)  
 (148) TDPNQKILYTSIYSSADMIVMN-YLSKLDGAKNVQIHGVGHI GLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 060-392 (azotof.)  
 (148) TDPNQKILYTSIYSSADMIVMN-YLSKLDGAKNVQIHGVGHI GLLMNSQVNSLIKEGLNGGGGLDTN  
 SEQ: 061-398 (firmus)  
 (148) TDPNQKILYTSIYSSADMIVMN-YLSKLDGAKNVQIHGVGHI GLLMNSQVNSLIKEGLNGGGHNTN  
 SEQ: 062-393 (badius)  
 (148) TDPNQKILYTSIYSSADMIVMN-YLSKLDGAKNVQIHGVGHI GLLMNSQVNSLIKEGLNGGGHNTN  
 SEQ: 063-Dc5h  
 (148) TDPNQKILYTSIYSSADMIVMN-YLSRLDGARNVQIHGVGHI GLLYSSQVNSLIKEGLNGGGGLNTN  
 SEQ: 064-Dc5f  
 (148) TDPNQKILYTSVYSSADLIVN-SLSRLIGARNLIIHGVGHI GLLTSSQVKGYYIKEGLNGGGGLNTN  
 SEQ: 065-Dc5c1  
 (151) TDPNQKILYTSVYSSADLIVN-SLSRLIGARNLIIHGVGHI GLLTSSQVKGYYIKEGLNGGGQNTN  
 SEQ: 066-Dc5a2  
 (151) TDPNQKILYTSVYSSADLIVN-SLSRLIGARNLIIHGVGHI GLLAASSQVKGYYIKEGLNGGGQNTN  
 SEQ: 067-Dc5l2  
 (151) TDPNQKILYTSVYSSADLIVN-SLSQFNWRKHPDPGVGHI GLLTSSQVKGYYIKEGLNGGGGLNTN  
 SEQ: 068-Sga  
 (148) TDPNQKILYTSVYSSADMIVMN-YLTKLDGAKNVQIHGVGHI GLLYSSQVNSLIKEGLNGGGGLNTN  
 SEQ: 069-Sgc  
 (148) TDPNQKILYTSVYSSADMIVMN-YLSKLDGAKNVQIHGVGHI GLLYSSQVNSLIKEGLNGGGGLNTN  
 SEQ: 070-Sgd  
 (148) TDPNQKILYTSVYSSADMIVMN-SLSRLIGARNVQIHGVGHI GLLTSSQVKGYYIKEGLNGGGGLNTN  
 SEQ: 071-Sgf  
 (148) TDPNQKILYTSVYSSADLIVN-SLSRLIGARNVQIHGVGHI GLLTSSLVKGYIKEGLNGGGQNTN  
 SEQ: 072-Sgh  
 (148) TDPNQKILYTSVYSSADLIVN-SLSRLIGARNVQIHGVGHI GLLTSSQVKGYYIKEGLNGGGGLNTN  
 SEQ: 073-Mt2b1  
 (151) TDPNQKILYTSVYKLSRSHCRQQLSFWNLQETVQIHGVGHI GLLTSSQVKGYYIKEGLNGGGGLNTN  
 SEQ: 074-H2a  
 (148) TDPNQKILYTSVYKLSRSHCRQQLSFWNLQETVQIHGVGHI GLLTSSQVKGYYIKEGLNGGGGLNTN

116

181

Figure 5c

75

1

SEQ: 075-1f15 (G2) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 076-3C12 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 077-3N19 (G2) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDRGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 078-G2.2 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 079-2F3 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 080-2F11 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 081-KV11 (6C7) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 082-KV6 (3A1) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 083-KV2 (2D1) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 084-N2.5 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 085-KV5 (2H6) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 086-3E5 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDRGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 087-G2.1 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 088-3H24 (G2) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 089-KV10 (4G6) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 090-KV12 (6D4) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 091-N2.2 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 092-N2.3 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 093-N2.1 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 094-KV4 (2E12) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 095-KV9 (4C6) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 096-7D6 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 097-3F3 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 098-2D11 (G2) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDRGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 099-3C23 (G2) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDRGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 100-G2.3 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDRGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 101-2A3 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 102-2F4 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 103-2B9 (G2) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 104-2C5 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 105-KV1 (2A6) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 106-2D13 (G2) (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 107-3C8 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

SEQ: 108-2D5 (1) EHNPFVVMVHGI GGASFN FAGIKSYLVSQGSRGKLYAVDFWMDKGTGNTYNNNGPVL SRFVQKVLDETGAKKVD IVAH

Figure 6a

150

76

SEQ: 075-1f15 (G2) (76) SMGGANTLYYIKNLDGGNKVENVTLGGTNRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 076-3C12 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 077-3N19 (G2) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 078-G2.2 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 079-2C3 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 080-2F11 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 081-KV11 (6C7) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 082-KV6 (3A1) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 083-KV2 (2D1) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 084-N2.5 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 085-KV5 (2H6) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 086-3E5 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGTNRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 087-G2.1 (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 088-3H24 (G2) (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 089-KV10 (4G6) (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 090-KV12 (6D4) (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 091-N2.2 (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 092-N2.3 (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 093-N2.1 (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 094-KV4 (2E12) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 095-KV9 (4C6) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 096-7D6 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 097-3F3 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 098-2D11 (G2) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 099-3C23 (G2) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRLTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 100-G2.3 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 101-2A3 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 102-2F4 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 103-2B9 (G2) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 104-2C5 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 105-KV1 (2A6) (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 106-2D13 (G2) (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 107-3C8 (76) SMGGANTLYYIKNLDGGNKVENVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

SEQ: 108-2D5 (76) SMGGANTLYYIKNLDGGNKIENVVTLGGANRSTTSKALPGTDPNQKILYTSIYSSADMIVMNYLSKLDGAKNVQI

Figure 6b

151 180

SEQ: 075-1f15 (G2) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 076-3C12 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 077-3N19 (G2) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 078-G2.2 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 079-2C3 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 080-2F11 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 081-KV11 (6C7) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 082-KV6 (3A1) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 083-KV2 (2D1) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 084-N2.5 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 085-KV5 (2H6) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 086-3E5 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 087-G2.1 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 088-3H24 (G2) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 089-KV10 (4G6) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 090-KV12 (6D4) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 091-N2.2 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 092-N2.3 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 093-N2.1 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 094-KV4 (2E12) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 095-KV9 (4C6) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 096-7D6 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 097-3F3 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 098-2D11 (G2) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 099-3C23 (G2) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 100-G2.3 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 101-2A3 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 102-2F4 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 103-2B9 (G2) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 104-2C5 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 105-KV1 (2A6) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 106-2D13 (G2) (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 107-3C8 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN  
 SEQ: 108-2D5 (151) HGVGHIGLLMNSQVNSLIKEGLNGGGGLNTN

Figure 6c

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